National Library of Canada
Bibliothèque nationale du Canada

Canadian Theses Service
Service des thèses canadiennes

Ottawa, Canada
K1A 0N4

NOTICE

The quality of this microform is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us an inferior photocopy.

Reproduction in full or in part of this microform is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30, and subsequent amendments.

AVIS

La qualité de cette microforme dépend grandement de la qualité de la thèse soumise au microfilmage. Nous avons tout fait pour assurer une qualité supérieure de reproduction.

S'il manque des pages, veuillez communiquer avec l'université qui a conféré le grade.

La qualité d'impression de certaines pages peut laisser à désirer, surtout si les pages originales ont été dactylographiées à l'aide d'un ruban usé ou si l'université nous a fait parvenir une photocopie de qualité inférieure.

La reproduction, même partielle, de cette microforme est soumise à la Loi canadienne sur le droit d'auteur, SRC 1970, c. C-30, et ses amendements subséquents.

NL-339 (r. 88/04) c

Canada

University of Alberta

SPECIATION, HYBRIDIZATION, AND PHYLOGENY OF PATROBUS GROUND BEETLES (COLEOPTERA: CARABIDAE)

by

Gregory Ray Pohl

a thesis

submitted to the faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

Department of Entomology

Edmonton, Alberta

Spring 1992

National Library of Canada
Bibliothèque nationale du Canada

Canadian Theses Service
Service des thèses canadiennes

Ottawa, Canada
K1A 0N4

The author has granted an irrevocable non-exclusive licence allowing the National Library of Canada to reproduce, loan, distribute or sell copies of his/her thesis by any means and in any form or format, making this thesis available to interested persons.

L'auteur a accordé une licence irrévocable et non exclusive permettant à la Bibliothèque nationale du Canada de reproduire, prêter, distribuer ou vendre des copies de sa thèse de quelque manière et sous quelque forme que ce soit pour mettre des exemplaires de cette thèse à la disposition des personnes intéressées.

The author retains ownership of the copyright in his/her thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without his/her permission.

L'auteur conserve la propriété du droit d'auteur qui protège sa thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

ISBN 0-315-73169-9

Canada

University of Alberta

Release Form

Name of Author: Gregory Ray Pohl

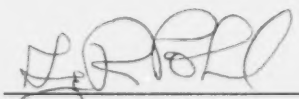
Title of Thesis: SPECIATION, HYBRIDIZATION, AND PHYLOGENY OF PATROBUS GROUND BEETLES (COLEOPTERA: CARABIDAE)

Degree: Master of Science

Year This Degree Granted: 1992

Permission is hereby granted to the University of Alberta Library to reproduce single copies of this thesis and to lend or sell such copies for private, scholarly or scientific research purposes only.

The author reserves other publication rights, and neither the thesis nor extensive extracts from it may be printed or otherwise reproduced without the author's written permission.



Gregory R. Pohl

c/o Department of Entomology
University of Alberta
Edmonton, Alberta
Canada T6G 2E3

Crowsnest Mountain, an important native medicine peak
in the heart of Patrobus country in southwestern Alberta.

University of Alberta
Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the faculty of Graduate Studies and Research for acceptance, a thesis entitled

SPECIATION, HYBRIDIZATION, AND PHYLOGENY
OF PATROBUS GROUND BEETLES (COLEOPTERA: CARABIDAE)

submitted by Gregory Ray Pohl in partial fulfillment of the requirements for the degree of Master of Science.

R. H. Gooding
Dr. Ronald H. Gooding

G. E. Ball
Dr. George E. Ball

A.R. Pal
Dr. A. Richard Palmer

Date: April 14/92

Dedicated to all those who struggle to retain a sense of humanity

in this dehumanizing quest for knowledge known as science.

ABSTRACT

Samples of Patrobus fossifrons (Eschscholtz) and P. stygicus Chaudoir were examined for morphological, electrophoretic, and ecological characteristics. A coastal morph and an inland morph of P. fossifrons are described, and their biogeographical history is discussed. P. stygicus was homogeneous across all populations for the features studied.

In allopatry, differences in all three character types indicate that P. stygicus and the two morphs of P. fossifrons are distinct. P. stygicus and inland P. fossifrons co-occurred at Cypress Hills, and in the Pincher Creek area. In the latter area, P. stygicus and inland P. fossifrons lived in different habitats. P. stygicus and coastal P. fossifrons co-occurred at Wauconda, Washington, with no evidence of habitat partitioning.

Indirect evidence of gene flow was examined in morphological and electrophoretic characters, and estimates of gene flow levels were calculated from electrophoretic data. Although two putative hybrids were found at Pincher Creek, gene flow estimates between P. stygicus and inland P. fossifrons were no higher there than in allopatry. A hybrid coastal P. fossifrons/P. stygicus specimen was found at Wauconda, which was at least an F_2, based on electrophoretic information. Gene flow between P. stygicus and coastal P. fossifrons was slightly higher in sympatry than in allopatry. In all cases, gene flow between P. stygicus and P. fossifrons was low enough that these taxa are able to remain genetically distinct, and they are ranked as separate species.

Although coastal and inland P. fossifrons can probably interbreed, estimates of gene flow between them were low enough that they are capable of diverging. They are probably species in the making; until the boundary between them is better understood, they remain grouped as a single species.

Electrophoretic data was gathered from all six North American Patrobus species, as well as from the European P. atrorufus and from two Diplous species, to test the previously-accepted phylogenetic positions of Patrobus species. The resulting reconstructed phylogeny suggested that P. fossifrons and P. stygicus may not be sister species, and that the subgenus Neopatrobus may not be monophyletic.

ACKNOWLEDGEMENTS

I would like to thank Ronald H. Gooding, my supervisor, for giving help and advice when I needed it, for not giving help and advice when I didn't need it, and for demonstrating patience above and beyond the call of duty. I also thank George E. Ball and A. Richard Palmer, for sitting on my supervisory committee, and for providing guidance and advice. Special thanks to George E. Ball, David W. Langor, and Felix A. H. Sperling for inspiration. Thanks also to my friends and fellow students, Tracy N. Kutash, Dean S. Mulyk, Daryl J. M. Williams, and Jack P. Wojcicki, for many hours of fascinating discussion. Specific thanks to Tracy for patience, emotional support, and for just being there through most of this; to Dean for friendship, collecting adventures, cross-country tours, and loads of computer help; to Daryl for friendship and intense discussions, and for going through it all first; and to Jack for a refreshing dose of craziness. Thanks also to Njeri Graham for long-distance support, and to Lori Reaney for an alternative view. Special thanks to The Same and Café Flesh for keeping the left side of my brain from consuming the right side of my brain.

Thanks to Brian Rolseth for technical help with electrophoresis, and Heather Nychka, who worked for me in the summer of 1989 under the *Women in Scholarship, Engineering, Science, and Technology* program. I would also like to thank most of the aforementioned, as well as Sheila Gooding, Jari Niemelä, Elfriede and Raymond Pohl, and John Spence, for collecting specimens for me at one time or other, and Gordon Pritchard at the University of Calgary and David Kavanaugh at the California Academy of Sciences, for loaning me museum specimens. Thanks also to Christian Klingenberg for endless help with multivariate statistics, and Brian V. Brown, Kenneth M. Fry, and Timothy G. Spanton for valuable discussions and help with computer programs.

Financial support was provided by the Natural Sciences and Engineering Research Council of Canada, through a postgraduate scholarship, and through an operating grant (no. A-3900) to Ronald H. Gooding, University of Alberta. Financial support was also provided by an Entomological Society of Canada postgraduate award, and a University of Alberta graduate faculty fellowship.

TABLE OF CONTENTS

Section	Page
1 INTRODUCTION	1
1.1 General Biology of Patrobus	1
1.2 Position of Patrobus in the Carabidae	1
1.3 Taxonomic History of Patrobus	2
1.4 Taxonomic Status of P. fossifrons and P. stygicus	3
1.5 Scope of This Study	3
2 MATERIALS, METHODS, AND PRELIMINARY DATA PROCESSING	6
2.1 Specimens Examined	6
2.2 Electrophoresis	7
2.2.1 Theory of Polyacrylamide Gel Electrophoresis	7
2.2.2 Materials and Methods	8
2.2.3 Results	11
2.2.4 Preliminary Data Processing	12
2.2.5 Summary	14
2.3 Morphological Examination	14
2.3.1 General Procedures	14
2.3.2 Qualitative Observations	15
2.3.3 Quantitative Observations	15
2.3.4 Summary	19
2.4 Ecological Data	19
2.5 Summary	19
3 EVALUATION OF SPECIES AND MORPHS	36
3.1 Introduction	36
3.2 Data Examined	36
3.3 Results	37
3.3.1 Character States of Allopatric Stocks of P. stygicus	37
3.3.2 Character States of Allopatric Stocks of P. fossifrons	39
3.4 Discussion	40
3.4.1 P. fossifrons Populations	40
3.4.2 P. stygicus Populations	44
3.4.3 Comparison of P. stygicus to P. fossifrons	45
3.5 Summary	46
4 EXAMINATION OF SYMPATRIC ZONES AND HYBRIDS	62

4.1 Introduction....62
4.2 Sympatric Zone Data Examined....62
4.3 Results and Interpretations....63
4.3.1 Pincher Creek, Alberta....63
4.3.2 Cypress Hills, Alberta....65
4.3.3 Wauconda, Washington....66
4.4 Discussion....67
4.4.1 Habitat Preferences....67
4.4.2 Hybrid Ancestries....68
4.4.3 Brachyptery of Hybrid Specimens....70
4.5 Summary....71
5 EVIDENCE AND ESTIMATES OF GENE FLOW....85
5.1 Theory....85
5.1.1 Indirect Evidence of Gene Flow....85
5.1.2 Electrophoretic-Based Estimates of Gene Flow....87
5.2 Materials and Methods....89
5.2.1 Discriminant Function Analysis Methods....89
5.2.2 Nei's Genetic Identity Calculation Methods....89
5.2.3 Heterozygosity and Symmetry Analysis Methods....90
5.2.4 Gene Flow Estimation Methods....91
5.3 Results....92
5.3.1 Morphological Introgression as Evidence of Gene Flow....92
5.3.2 Electrophoretic Introgression as Evidence of Gene Flow....93
5.3.3 Heterozygosity and Symmetry....93
5.3.4 Estimates of Gene Flow....95
5.3.5 Summary of Results....98
5.4 Discussion....99
5.4.1 P. fossifrons versus P. stygicus....99
5.4.2 Status of P. fossifrons morphs....101
5.4.3 Homogeneity of P. stygicus....102
5.4.4 Other Questions....102
5.5 Summary....103
6 RELATIONSHIPS IN THE GENUS PATROBUS....117
6.1 Introduction....117
6.2 Theory....117
6.2.1 Morphological versus Electrophoretic Characters....117

6.2.2 Genetic Distance Analysis 118
6.2.3 Discrete Character Analysis 119
6.2.4 Summary 121
6.3 Materials and Methods 121
6.3.1 Specimens Examined 121
6.3.2 Distance Analysis Procedure 122
6.3.3 Discrete Character Analysis Procedure 123
6.4 Results 123
6.4.1 Distance Analysis 123
6.4.2 Discrete Character Analysis 124
6.5 Discussion 124
6.5.1 Age and Status of the Taxa 124
6.5.2 Evolutionary Hypotheses Within Patrobus 125
6.6 Summary 129
7 Conclusions 135
7.1 Answers to Questions 135
7.2 General Conclusions 136
References Cited 138
Appendix #1. Collection Locality Data 145
Appendix #2. Chemical Suppliers 148
Appendix #3. Details of Enzyme Stains 149
Appendix #4. Electrophoretic Data 151
Appendix #5. Morphological Data 163
Vita 180

LIST OF TABLES

Table		Page
2.1	Enzyme abbreviations and stain recipe references	21
2.2	Cases of Hardy-Weinberg disequilibrium in electrophoretic data	21
2.3	Chi-squared tests of electrophoretic data for heterogeneity among different days' samples	22
2.4	Pooled samples not conforming to Hardy-Weinberg equilibrium	22
2.5	Allele frequencies of Patrobus and Diplous population samples	23
2.6	Genotypes of single specimen samples of Patrobus and Diplous	29
3.1	Qualitative morphological characteristics of allopatric populations	47
3.2	Nei's (1978) unbiased genetic identities between allopatric populations	48
3.3	Summary of characteristics of 'coastal' and 'inland' Patrobus fossifrons, and P. stygicus, in allopatry	48
4.1	Comparison of qualitative morphological characters in sympatric and pooled allopatric populations	72
4.2	Electrophoretic comparison of sympatric and pooled allopatric populations	73
5.1	Test of a sample of 16 Rock Pond 'inland' P. fossifrons males for significance of bilateral variances	105
5.2	Nei's (1978) unbiased pairwise genetic identities and distances between sympatric and pooled allopatric populations	106
5.3	Heterozygosities of Patrobus population samples	107
5.4	Bilateral variances (symmetries) of wing measurements, for large samples of Patrobus	107
5.5	F-tests of bilateral variances of wing measurements of large samples of Patrobus	108
5.6	Bilateral variances of homozygotes and heterozygotes at various loci, for large samples of Patrobus	109
5.7	Estimates of gene flow between Patrobus species and morphs	110
5.8	Wright's (1951) F-statistics of electrophoretic data for species and morphs of Patrobus	111
5.9	Slatkin's (1985a) pairwise Nm estimates between Patrobus fossifrons populations	112
5.10	Slatkin's (1985a) pairwise Nm estimates between Patrobus stygicus populations	113

6.1 Pairwise genetic comparisons of Patrobus and Diplous population samples 131
6.2 Binary electrophoretic codings for Patrobus and Diplous species and morphs 132

LIST OF FIGURES

Figure **Page**

1.1 Reconstructed phylogeny of the supertribe Patrobitae 5
1.2 Reconstructed phylogeny of North American Patrobus species 5

2.1 Collection sites of Patrobus fossifrons and P. stygicus in western North America 30
2.2 Collection sites of Patrobus fossifrons and P. stygicus in the southeastern British Columbia and southwestern Alberta 31
2.3 Examples of electrophoretic gels stained for AO, ODH, EST 32
2.4 Examples of electrophoretic gels stained for APK, PGI 32
2.5 Examples of electrophoretic gels stained for EST, HK, PGM 33
2.6 Elytral microsculpture micrographs 33
2.7 Penis apices (right side view) 34
2.8 Wing of Patrobus fossifrons, showing measurements taken 34
2.9 Pronotal length versus pronotal width, for all Patrobus fossifrons and P. stygicus specimens 35
2.10 Tip of antenna of Patrobus fossifrons, showing measurements taken 35

3.1 Pronotal length of females from allopatric populations 49
3.2 Pronotal length of males from allopatric populations 50
3.3 Pronotal width minus length of females from allopatric populations 51
3.4 Pronotal width minus length of males from allopatric populations 52
3.5 Antenna tip length of females from allopatric populations 53
3.6 Antenna tip length of males from allopatric populations 54
3.7 Ninth antennomere squareness of females from allopatric populations 55
3.8 Ninth antennomere squareness of males from allopatric populations 56
3.9 Relative length of R_1 wing vein in allopatric populations 57
3.10 Relative width of wing wedge cell in allopatric populations 58
3.11 Typical pronota of Patrobus fossifrons and P. stygicus 59
3.12 Typical antenna tips of 'coastal' Patrobus fossifrons, 'inland' P. fossifrons, and P. stygicus 60
3.13 Typical flight wings of Patrobus fossifrons and P. stygicus 61

4.1 Patrobus fossifrons and P. stygicus sites in the Pincher Creek area of southwestern Alberta 74

4.2 Pronotal length of females from sympatric populations 75
4.3 Pronotal length of males from sympatric populations 76
4.4 Pronotal width minus length of females from sympatric populations 77
4.5 Pronotal width minus length of males from sympatric populations 78
4.6 Antenna tip length of females from sympatric populations 79
4.7 Antenna tip length of males from sympatric populations 80
4.8 Ninth antennomere squareness of females from sympatric populations 81
4.9 Ninth antennomere squareness of males from sympatric populations 81
4.10 Relative length of R_1 wing vein length in sympatric populations 82
4.11 Relative width of wing wedge cell in sympatric populations 83
4.12 Possible ancestries of the Wauconda specimen of mixed 'coastal' Patrobus fossifrons (Pfs-c) / P. stygicus (Pst) stock 84

5.1 Multivariate comparison of Patrobus stygicus and 'inland' P. fossifrons 114
5.2 Multivariate comparison of Patrobus stygicus and 'coastal' P. fossifrons 115
5.3 Heterozygosity versus bilateral variance of wing measurements in Patrobus population samples 116

6.1 Phenogram of Patrobus and Diplous populations, based on Cavalli-Sforza and Edwards (1967) arc distances 133
6.2 Reconstructed phylogeny of Patrobus species, using absolute presence / absence of alleles as binary character states 134
6.3 Reconstructed phylogeny of Patrobus species, using 5% criterion of presence / absence of alleles as binary character states 134

LIST OF ABBREVIATIONS USED

ALN.....antenna tip length
AO..... aldehyde oxidase
APK.....arginine phosphokinase
ASQ.....ninth antennal segment squareness
EST-1.....esterase-1
EST-3.....esterase-3
HK..... hexokinase
I2..... secondary sclerotized island of hindwing
NAD ..... ß-nicotinamide adenine dinucleotide
ODH .....octanol dehydrogenase
Pfs..... P. fossifrons
Pfs-c .....'coastal' morph of P. fossifrons
Pfs-i..... 'inland' morph of P. fossifrons
PGI.....phosphoglucose isomerase
PGM.....phosphoglucomutase
PLN.....pronotal length
Pst..... P. stygicus
PWL ..... pronotal width minus length
R1 ..... relative length of R_1 wing vein
R1.1.....length of the sclerotized area of the fused costa, subcosta, and radius
R1.2.....length of the R_1 vein extending from R1.1
TEMED..... N,N,N,N'-tetramethylene-diamine
W..... relative width of the wedge cell on the hind wing
W1 .....length of the cubital vein between the two cubito-anal cross veins
W2 ..... width of the wedge cell

1. INTRODUCTION

1.1 General Biology of Patrobus

Adults of Patrobus Dejean are structurally unspecialized carabid beetles, 8 to 15mm long. Their distribution is holarctic.

Six species of Patrobus live in North America. P. septentrionis Dejean and P. foveocollis Eschscholtz range across the northern part of the continent, south as far as Colorado. P. longicornis Say is the most southern, occurring across the central and eastern United States, and North into southern Canada. P. lecontei Chaudoir lives in central Canada, south into the Rocky Mountain states. P. fossifrons (Eschscholtz) and P. stygicus Chaudoir are more or less parapatric, with their combined ranges extending across northern North America south to Colorado. P. fossifrons lives primarily West of the Rockies, while P. stygicus lives primarily East of them. The ranges of these two species are discussed in more detail below. All six of these species have been collected in Alberta.

The habitats of North American Patrobus species are moist areas such as damp forests and meadows, and pond and stream margins. P. stygicus and P. fossifrons are the most hygrophilous, living a semi-aquatic lifestyle in sedge clumps and under debris at pond margins. Patrobus beetles are generalized predators and scavengers.

1.2 Position of Patrobus in the Carabidae

According to Lindroth (1961), the most closely related genus to Patrobus is Platypatrobus Darlington. These genera, along with Diplous Motschulsky, make up the tribe Patrobini. The Patrobini and its sister tribe the Deltomerini (containing the genera Platidiolus Chaudoir, Deltomerus Motschulsky, and Paradeltomerus Apfelbeck) make up the supertribe Patrobitae.

Another group in the Patrobitae, Penetretus Motschulsky, cannot be placed in either tribe with certainty. Ledoux (1984) states that Penetretus is clearly monophyletic, but because the diagnostic characters of the tribes are not reliable, species within the genus would be divided between Patrobini and Deltomerini. Currently, the genus is *incertae sedis*.

According to Kryzhanovskiy (1976), members of the supertribe Patrobitae are similar to members of the Broscitae, Psydritae, and Trechitae, but they also share some characteristics with the Pterostichitae. The phylogenetic relationships among these supertribes are currently unresolved, so in Kryzhanovskiy's classification, Patrobitae has

simply been placed between the Trechitae and the Pterostichitae. The current state of classification of the Patrobitae is summarized in Figure 1.1.

1.3 Taxonomic History of Patrobus

The genus name Patrobus was first published by Dejean (1821). The type species, P. atrorufus (Stroem), had been around for a long time as Carabus rufipes before it was placed into Patrobus. Darlington (1938) points out that, technically, Patrobus ought to be considered as a junior synonym of Calathus, because the particular specimens of Carabus rufipes that Dejean examined were actually misidentified Calathus, and not Patrobus at all. However, so much literature about "Patrobus" exists that the name has been kept, despite the technicality.

The currently-accepted relationships of North American species of Patrobus are based primarily on Darlington (1938). His phylogenetic conclusions were based on external morphological characters, detailed examination of the male genitalia, and on geographic distribution. He also examined female genitalia, but did not find any useful characters there. Darlington reconstructed the phylogeny of North American species of Patrobus. He also concluded that members of the genus Patrobus likely originated in Asia, and then speciated and migrated into Europe and North America. Although he erected the subgenera Neopatrobus, Geopatrobus, and Patrobus *sensu stricto*, he did not hypothesize what their relationships might be.

Kühnelt (1941) examined the worldwide Patrobus species, and erected six species groups. He postulated that Patrobus first differentiated into a European and an Asian assemblage. The European assemblage (corresponding to Darlington's Patrobus *sensu stricto*) diverged into three species groups, and the Asian assemblage diverged into an Asian group and two other groups corresponding to Darlington's Geopatrobus and Neopatrobus subgenera. Kühnelt's ideas of these relationships were based on the structure of the male genitalia, and on present-day geographic distributions.

Lindroth's (1961) treatment of the Carabidae of Canada and Alaska included the North American patrobines. He agreed with Darlington's appraisal of the relationships in the group, but changed the status of some of Darlington's subspecies. He did not recognize Darlington's subspecies of P. lecontei and P. foveocollis, and he changed the status of P. fossifrons and P. stygicus (see Section 1.4 for details).

Carter (1971) studied the ecology of Patrobus in central Alberta. He accepted Darlington's phylogeny, and fitted the habitat preferences of Patrobus species to this

phylogeny. His study was not a test of the phylogeny, but it did provide some corroborative evidence for previous ideas.

Recent description of many new Asian patrobines has left the classification of Patrobus in disarray. The group Minypatrobus Ueno was considered by Habu (1972) to be a subgenus of Patrobus, but Lindroth (1961) stated that it was related to Platidiolus, in the sister tribe to the Patrobini, the Deltomerini. Habu (1972, 1973, 1976) described several Asian species in Minypatrobus and in another new subgenus, Apatrobus Habu. Apatrobus is a previously unrecognized group, and, according to Nakane (1978), does not correspond to Kühnelt's Asian species group, which is no longer recognized.

The current reconstruction of the phylogeny of Patrobus (Figure 1.2) summarizes the contributions of the aforementioned workers, and indicates current uncertainties.

1.4 Taxonomic Status of P. fossifrons and P. stygicus

Darlington (1938) considered P. stygicus to be a subspecies of P. fossifrons; P. fossifrons stygicus Chaudoir. Darlington also split the present-day P. fossifrons on the basis of their wing development. He recognized the brachypterous populations of the Aleutian Islands as P. fossifrons fossifrons (Eschscholtz) (type locality Unalaska Island), and the remaining wing-dimorphic populations as P. fossifrons dimorphicus Darlington (type locality near Victoria, Vancouver Island). Though he considered these three taxa to be subspecies, Darlington noted that more information might prove P. fossifrons stygicus to be a full species. This is exactly what Lindroth determined in 1961, and this is how they are treated today. As well, Lindroth did not recognize Darlington's P. fossifrons fossifrons as a subspecies of P. fossifrons. He could not find any difference between it and Darlington's P. fossifrons dimorphicus, other than the fact that P. fossifrons dimorphicus had at least a few long-winged individuals in every population, so he grouped the two as P. fossifrons.

1.5 Scope of This Study

The recent discovery (Ball, unpublished) of possible P. fossifrons-stygicus intergrades in southern Alberta has cast the status of these taxa as separate species in doubt. In 1987, I tackled this problem as an undergraduate project in Entomology, under the guidance of G. E. Ball. That study provided more questions than answers, so in 1988 I expanded it into this MSc. thesis project. The questions I will attempt to answer are the following:

1. Is the current taxonomic status of P. stygicus and P. fossifrons as separate species correct?
2. Is there any variation within these taxa (for example the reduced-wing versus wing-dimorphic populations of P. fossifrons) that ought to be recognized taxonomically?
3. Does hybridization occur between these taxa? What conditions allow it to occur or prevent it from occurring?
4. Are the currently accepted phylogenetic positions of P. fossifrons and P. stygicus within Patrobus correct?

Most previous taxonomic work on Patrobus has involved morphological and ecological characters. This is suitable for many problems, but it does not yield much information when very similar species such as P. fossifrons and P. stygicus are compared. In this study, morphological and ecological data are compared and contrasted with electrophoretic data. By using these different data types the biases of each becomes evident, and a truer picture should emerge.

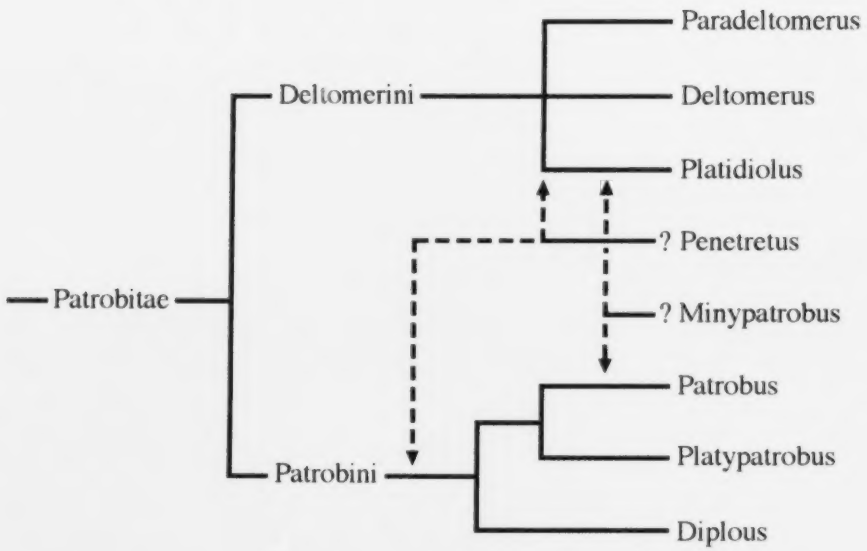


Figure 1.1: Reconstructed phylogeny of the supertribe Patrobitae. Based on Kurnakov (1960), Lindroth (1961), and Kryzhanovskiy (1976).

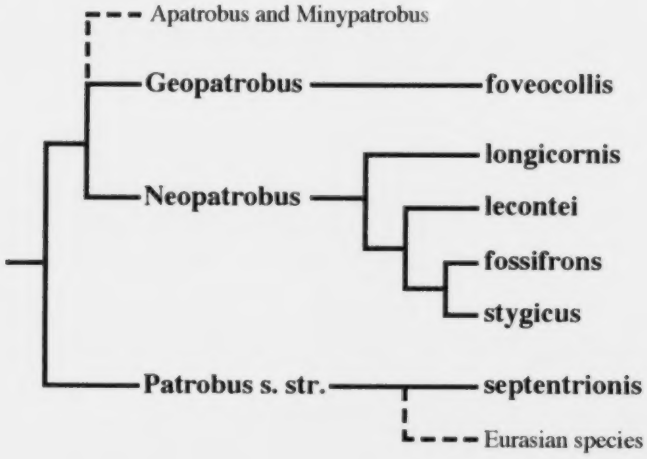


Figure 1.2: Reconstructed phylogeny of North American Patrobus species. Dashed lines indicate probable positions of Palearctic taxa. Based on Darlington (1938), Kühnelt (1941), Lindroth (1961), and Nakane (1985).

2. MATERIALS, METHODS, AND PRELIMINARY DATA PROCESSING

2.1 Specimens Examined

In this study, 520 pinned and 617 live specimens were examined. All six North American species of Patrobus were examined, as well as P. atrorufus from Europe. Diplous aterrimus Dejean and D. californicus Motschulsky were used as outgroups. Collection localities of P. fossifrons and P. stygicus are mapped in Figures 2.1 and 2.2. Samples of P. septentrionis and P. foveocollis were collected at P. fossifrons and P. stygicus sites. P. lecontei was collected at P. fossifrons and P. stygicus sites, and at Bennett and Frith Ponds in the Pincher Creek area. P. longicornis was collected at the North Saskatchewan River near Edmonton, and P. atrorufus was collected near Helsinki, Finland. D. aterrimus samples were collected at the North Saskatchewan River near Rocky Mountain House, the Oldman River near Pincher Creek, and the Waterton River near the Waterton Dam. D. californicus were collected at the Similkameen River near Hedley. Detailed label information for all examined specimens appears in Appendix #1.

Pinned beetles were from my personal collection, and from the following institutions, with names of curators in parentheses:

BDUC: Biology Department, University of Calgary, Calgary, Alberta T2N 1N4, (G. Pritchard);

CAS: California Academy of Sciences, San Francisco, California 94118, (D.H. Kavanaugh);

UASM: University of Alberta, Department of Entomology, Strickland Museum, Edmonton, Alberta T6G 2E3, (G.E. Ball).

Live specimens were collected in western North America in 1988, 1989, and 1990. Most of them were obtained by treading sedge hillocks and looking under debris around pond margins. Exceptions were P. longicornis, D. aterrimus, and D. californicus specimens, which were found under rocks and debris along river margins, and P. atrorufus specimens, which were collected in forest pitfall traps by J.R. Spence and J. Niemelä.

Specimens were kept on cool damp moss in petri dishes. Purina® brand dry cat food was provided as food. Once in the laboratory, each specimen was identified, sexed, measured, given a unique alphanumeric identification code, and then stored frozen at -70°C

until further analysis. Afterwards, beetles, wings (mounted on glass slides), and other beetle parts (glued to cards) were deposited in the Strickland Museum, in the Department of Entomology at the University of Alberta.

Most of the live material was sampled for both morphological and electrophoretic characters, but pinned specimens could be sampled only for morphological characters. Beetles with incomplete data were included in analyses whenever possible.

2.2 Electrophoresis

2.2.1 Theory of Polyacrylamide Gel Electrophoresis

Electrophoresis is a process which separates protein molecules on the basis of their electrical charge, size, and shape, by using an electrical field to force them through a gel. Compounds are separated on the basis of charge, since positively-charged compounds (cations) migrate toward the cathode, and negatively-charged compounds (anions) migrate toward the anode. Electrophoretic filtering properties thus depend on the strength of the electrical current, and on the pH of the gel (the charges of the proteins depend on the pH of the medium they are in). The gel also physically sieves compounds, to separate them on the basis of size and shape. All of this is expressed in the following formula:

[1.1] $$U = Qd/4\Pi r^2 n$$

where **U** = the rate of movement of the molecule, **Q** = net molecular charge, **r** = radius as an indicator of molecular size and shape, **n** = the viscosity of the gel, and **d** = the strength of the electrical field applied to the gel (Murphy *et al.* 1990).

In polyacrylamide gel electrophoresis, crystalline acrylamide is polymerized into a chain by persulfate, riboflavin, and N,N,N,N'-tetramethylene-diamine (TEMED), which act as catalysts. By including a small amount of N,N'-methylene-bis-acrylamide, crosslinks are put into the chain, to form a mesh. The tightness of the mesh, and thus the filtering properties, depend on the acrylamide concentration in the gel, and the percent of acrylamide that is bis-acrylamide.

The buffers used in electrophoresis make an electrical connection between the electrodes and the gel, maintain a constant pH (and thus keep relative electrical charges constant), and stabilize the compounds being electrophoresed. The buffer pH is generally kept on the alkaline side of the isoelectric point of the electrophoresed compounds, so they will be negatively charged, and migrate through the gel to the positive electrode.

In biology, enzymes can be studied electrophoretically. They usually occur in very small quantities, but their presence can be determined by using them to catalyze a reaction yielding a highly visible product. Resulting bands in different positions are termed electromorphs. An assumption of enzyme electrophoresis is that different electromorphs are coded by different alleles. This is generally a safe assumption, but not all alleles result in unique electromorphs. Selander (1976) pointed out that 16 of the 20 amino acids are electrically neutral within the pH range of most electrophoretic analysis. Thus changes in these neutral amino acids would not alter the charge of a protein. Some of these alleles can be separated on the basis of their conformation alone, but many alleles are missed by standard electrophoretic procedures. King and Wilson (1975) calculated that only 27% of point mutations are electrophoretically detectable. Electromorphs, then, are not exactly the same as alleles, but they are analyzed in the same manner.

Grouping several alleles as the same electromorph reduces the level of detail visible in a study; we are not seeing all the variation which exists, so the picture becomes 'grainy'. However, the statistical analyses are still sound; alleles can be combined, as long as no allele is split into two or more electromorphs.

2.2.2 Materials and Methods

Apparatus and Gels

Electrophoretic procedures are based on those of Rolseth and Gooding (1978). See Appendix #2 for sources of chemicals. Electrical equipment included a Sorvall® RC-5B centrifuge, a Hewlett-Packard® 6448B power supply, a water cooling unit, and two warm water baths. The electrophoretic apparatus was built by B. M. Rolseth and M. G. MacIntyre, and is similar to the commercially-available Bio-Rad® system. It has a 60 x 140 x 115mm cooling chamber, to which two sheets of glass are clamped to form an upper buffer chamber with a 140 x 160 x 0.75mm gel chamber on either side. A large Plexiglas® tank houses this entire apparatus, and forms the lower buffer chamber.

Electrophoresis stock solutions were based on those of Rolseth and Gooding (1978), with some modifications. They were prepared with glass-distilled water as follows:

buffer A89: 24ml 1M HCl, 18.1g Trizma®-base, and 0.12ml TEMED/100ml H_2O, adjusted to pH 8.9;

buffer A82, pH 8.2: 60ml 1M HCl, 18.1g Trizma®-base, and 0.12ml TEMED/100ml H_2O, adjusted to pH 8.2;

buffer B: 25.6ml 1M H_3PO_4, 5.7g Trizma®-base, and 0.46ml TEMED/100ml H_2O, adjusted to pH 6.7;

C7 acrylamide: 27.51g acrylamide, and 0.49g N,N'-methylene-bis-acrylamide/100ml H_2O, filtered through a vacuum funnel;

C9 acrylamide: 35.58g acrylamide, and 0.42g N,N'-methylene-bis-acrylamide/100ml H_2O, filtered through a vacuum funnel;

solution D: 24g acrylamide, and 5g N,N'-methylene-bis-acrylamide/100ml H_2O, filtered through a vacuum funnel;

solution E: 4.0mg riboflavin/100ml H_2O, filtered;

solution G: 0.14g ammonium persulfate/100ml H_2O.

The gel slabs consisted of a 100mm high separating gel (25% A solution, 25% C solution, and 50% G solution), under a 40mm high stacking gel (65.5% H_2O, 16.5% D solution, 15% E solution, and 12.5% B solution). Twenty sample slots (0.75 x 4 x 30mm) were formed in each gel by inserting a teflon comb in the stacking gel before it set.

Sample Preparation and Analysis

Prior to electrophoresis, beetles were stored at -70°C. After thawing, elytra, wings, legs, and antennae were removed and saved for further study. Homogenates were prepared by grinding the head and thorax in homogenizing solution (20mg DL-dithiothreitol, 150mg polyvinyl-pyrrolidone, 0.5ml B solution, and 3.5ml distilled H_2O). The head and thorax of each beetle was homogenized in 70μl, and each abdomen was homogenized in 140μl.

Samples were centrifuged at 76000G for six to eight minutes, and the supernatant portion loaded into the gel slots with a microsyringe. Tank buffer (57.6g glycine, 12.0g Trizma®-base/4L H_2O) was then put into the upper and lower buffer chambers, and two drops of bromophenol blue marker dye was added to the upper buffer chamber. The cathode was connected to the upper buffer chamber, and the anode was connected to the lower buffer chamber. An electrical current of 25mAmps per gel was applied for approximately three hours, during which time the samples and gels were kept cool by circulating 0°C water through the cooling chamber.

Staining

At the end of electrophoresis, the gels were removed from the apparatus, and stained according to various staining recipes. Stain buffers were prepared as follows:

200mM tris buffer, pH 8.0: 30.84g Trizma® hydrochloride, and 17.76g Trizma® base/1.0L H_2O, adjusted to pH 8.0;

50mM tris buffer, pH 7.2: 7.02g Trizma® hydrochloride, and 0.67g Trizma® base/1.0L H_2O, adjusted to pH 7.2;

200mM phosphate buffer, pH 6.0: 123ml 0.2M Na_2HPO_4, and 877ml 0.2M NaH_2PO_4, adjusted to pH 6.0.

During staining, gels were incubated at 37°C, unless otherwise noted. After bands appeared, the enzymes were denatured by placing gels and overlays in 7% acetic acid. Gels were then scored, and stored in the dark in plastic bags. Cellulose acetate overlays were stored in the dark in paper envelopes.

Gels were stained successfully for eight enzymes (Table 2.1). Details of the procedures appear in Appendix #3. This appendix also contains a list of 19 other enzyme stains which were attempted unsuccessfully. Throughout the thesis I follow the convention of Gooding and Rolseth (1979) and refer to enzymes with a capitalized abbreviation, and to the enzyme locus with an italicized abbreviation.

For each enzyme, the Rf values were calculated as the distance the bands moved during electrophoresis, divided by the distance the buffer front (as indicated by the bromophenol blue dye) moved towards the anode.

Any bands for which the mobility was questionable (usually due to technical problems such as smearing and denaturation) were deleted from the analysis. Assigning of electromorphs was based on banding patterns of many individuals run over many days, and was verified by B. M. Rolseth. Electromorphs of very similar mobilities were rerun in adjacent slots on a gel, whenever possible, to ensure that they were truly different.

Rf values were corrected for slight fluctuations in gel concentration and pH, by using the predominant electromorph for each locus, as follows: First, the average Rf of the predominant allele was determined using as many gels as possible. Then, the 'shift factor' for each gel was calculated; the average Rf of the predominant allele across all gels divided by the Rf of the predominant allele on that gel. Finally all the bands on the gel were multiplied by this factor. Bands were then assigned to various electromorph classes, based on these corrected Rf values.

Electromorphs which were so close together that they could not be distinguished accurately on a gel (approx. Rf difference of 0.005) were combined as a single electromorph. Each recognized electromorph is assumed to be coded by a unique allele. For each locus, electromorphs were lettered in order of increasing Rf value, to represent these alleles.

2.2.3 Results

Figures 2.3 to 2.5 illustrate representative gels and banding patterns resulting from the staining procedures described above. Tables of electromorph codings, for individual beetles, appear in Appendix #4.

All eight enzymes were monomers, except for ODH and PGI, which were dimers. Based on banding patterns, all eight enzymes appear to be products of autosomal loci in the beetles examined.

Band Reading Difficulties

Coding of electromorphs of AO was particularly problematic. Stained bands were doubled in several species (P. stygicus, P. septentrionis, P. atrorufus, and sometimes P. foveocollis). One of these bands is likely a conformer, while the other is the actual electromorph. This 'doubling' of bands was noted by Johnson (1978), for alcohol dehydrogenase of Drosophila mojavensis. In that study, experiments revealed that the slow band was the true electromorph, and the fast band was due to binding of ß-nicotinamide adenine dinucleotide (NAD) to the enzyme.

NAD binding was not the cause of doubling in Patrobine AO, since addition or deletion of NAD at the homogenizing step did not alter the appearance of the conformers. However, some other factor is likely altering the mobility of some of the enzyme molecules in a similar manner. It is not known what this compound could be.

P. stygicus had some variation within these doubled bands (Figure 2.3). The distance between the two bands (conformer and electromorph) was variable, as was the position of the bands on the gel. This variation is similar to the situation described by Johnson (1978). He reported that the NAD-induced conformers of different electromorphs were often in similar positions. As well, he noted that hidden variation existed; electromorphs were often coded for by several alleles, all with the same mobility. These alleles could be distinguished because they had different levels of reactivity with NAD, and thus produced conformers in different positions. Thus it appears that different electromorphs can have the same conformer, and the same electromorph can have different conformers, creating very complex banding patterns. This results in various combinations of slow and fast bands, and is likely what is occurring in Patrobus AO.

Unfortunately, the hidden variation could not be deciphered in the current study. All P. stygicus bands were close together (Rf values between 0.275 and 0.310), and distinguishable from bands in most other species. Only alleles of P. septentrionis and P.

foveocollis individuals were within the range of bands of P. stygicus AO. For this study, the P. stygicus bands are grouped together, along with the P. septentrionis and P. foveocollis bands. The resulting electromorph is given an Rf range rather than a specific value. Doing so means ignoring some variation, but this will be no different from the hidden variation characteristic of most every enzyme system.

P. atrorufus also had doubled bands at Rf 0.315 and 0.330. Since this overlaps with bands in other species, (P. fossifrons and P. lecontei at Rf 0.325) they were all coded as the same electromorph in this study, again with an Rf range rather than a specific value.

Doubling of bands was also present in EST-3, in P. stygicus, P. septentrionis, P. foveocollis, P. lecontei, D. aterrimus, and occasionally in D. californicus (the latter more often in head-thoracic than in abdominal samples). The two bands were of equal intensity, and, unlike AO, the relative distance between them was always the same (Rf difference of 0.032). In this study, the slower band is considered to be the actual electromorph, so its Rf value is used.

These conformers often were in the positions of other electromorphs of the same species, resulting in double, triple, and quadruple bands, depending on the allele combination. Johnson (1977) described a situation similar to this, where bands were evenly spaced, as predicted by Ohta and Kimura's (1973) ladder model of enzyme variation (see Chapter 6 for details). This is probably due to some unknown factor acting on the enzyme molecules, altering their conformations in a manner similar to changes caused by genetic differences. The result is that the conformer ends up with the same conformation as another electromorph, and thus the same electrophoretic mobility. This in spite of the fact that they may have amino acid differences.

In spite of these conformers, it was still possible to score the various electromorphs of EST-3, once these complex banding patterns were understood.

2.2.4 Preliminary Data Processing

This section involves a series of tests applied and analyzed sequentially. Subsequent tests depend on results of the previous tests. Therefore, methods and results of each test are discussed together in this section, so that the text follows a logical progression.

Hardy-Weinberg Tests for Equilibrium

Initially, beetles were categorized according to species, collection site, and date of collection. After coding of alleles, all of these groups of specimens which contained at least five individuals were tested for deviation from Hardy-Weinberg equilibrium. For groups

with at least 20 individuals, a chi-squared test was carried out. For groups with fewer than 20 individuals, a Fisher exact probabilities test was used instead of a chi-squared test, to correct for small sample sizes.

Over 94% of the tests indicated that the groups were in equilibrium, using a 95% confidence interval. Of 139 tests made on 31 population samples, only eight (5.8%) of the tests did not conform to equilibrium. This is what was expected; about five percent of tests would not conform to Hardy-Weinberg equilibrium when using a 95% confidence interval. Groups which were not in equilibrium were examined (Table 2.2), to see if they fit any pattern. None was discerned, so it was concluded that the populations studied were randomly-breeding, and that any disequilibrium was not biologically significant.

Temporal Comparisons

Several sites were sampled more than once over the three year collection period. At each of these sites, chi-squared tests for homogeneity among samples were carried out at each locus, for all populations having at least five individuals scored for the particular locus. Chi-squared values and degrees of freedom were then summed across all variable loci, to determine the probability of the different samples being homogeneous. Summaries of these tests appear in Table 2.3. At P. fossifrons and P. stygicus sites, samples collected on different days were not significantly different, except for slight differences between the two samples collected at Hinton. It is concluded that, in general, there is little significant temporal variation in allele frequencies within a single season, nor over three seasons. For each of these multi-sampled sites, the various days' samples were pooled together.

Separate days' samples of P. lecontei from the same sites were combined also, as were those of P. septentrionis. These samples were too small for a chi-squared test for heterogeneity. The test of D. aterrimus samples from Rocky Mountain House showed that there was some temporal variation in this population. Despite this fact, the two days' samples were combined. Since D. aterrimus is an outgroup in this study, a small amount of substructuring within its populations will not seriously affect its use in the analysis of relationships within Patrobus.

Pooled Sample Hardy-Weinberg Tests

Hardy-Weinberg tests were carried out on resulting pooled samples, similar to the tests of unpooled groups described above. Deviations from equilibrium appear in Table 2.4. In Chain and George populations, these deviations occurred for the same reasons as deviations in unpooled samples. In all other instances of significant deviation from equilibrium, the deviations were present as trends in the unpooled samples, but they were

not significant. It is concluded that the deviations in pooled samples are due to random factors, not to the pooling of samples.

A summary of allele frequencies, with samples pooled as described above, appears in Table 2.5. Populations containing only single specimens appear in Table 2.6. Details of individual genotypes can be obtained from Appendix #4.

2.2.5 Summary

Patrobine populations were sampled for eight electrophoretic loci; *Ao*, *Apk*, *Est-1*, *Est-3*, *Hk*, *Odh*, *Pgi*, and *Pgm*. No significant deviation from Hardy-Weinberg equilibrium was found within samples. Little significant temporal variation was found between samples taken over the course of a summer, nor between samples taken over three summers. Different days' samples were pooled together for each species, at each site. Resulting pooled samples were generally in Hardy-Weinberg equilibrium.

2.3 Morphological Examination

2.3.1 General Procedures

Morphological data were gathered from both pinned and electrophoresed beetles, which were identified using Lindroth's (1961, 1974) keys. Identities of intermediate specimens were checked using electrophoretic characters discussed in Chapter 3.

When necessary, wings and genitalia were removed after softening specimens in warm, soapy water. Genitalia were placed in microvials and re-associated with the pinned specimens from which they were removed. Wings of pinned specimens were extended and glued to cards, while those of electrophoresed specimens were permanently mounted on glass slides.

Several possible differences between P. fossifrons and P. stygicus were noted by Darlington (1938), by Lindroth (1961), and by me. These characteristics were observed and measured through a Wild® M5 binocular microscope. Distances were measured using an ocular scale, and later converted to millimeters. Specimens were re-examined randomly throughout the course of measuring, to ensure that observations and measurements were consistently taken for all specimens. Measurements were generally found to be accurate to within one micrometer unit (±40μm @25X, and ±20μm @50X). Details are as follows:

2.3.2 Qualitative Observations

1. Shape of units of elytral microsculpture. Specimens were observed at 50X magnification, and coded as '1' (completely isodiametric) through '5' (completely transverse) See Figure 2.6 for examples of character states '1' and '5'.

2. Shape of the median lobe of male genitalia. Curvature of the tip and development of the barb near the tip was variable. These characters were part of the same trend in variation; the barb was larger on specimens with a more curved tip. They were coded as '1' (straight, spoonlike, with a very small barb) through '5' (strongly curved and strongly barbed, like a fish-hook). See Figure 2.7 for examples of character states '1' and '5'.

3. Development of the hindwing. Wings were either reduced and useless for flight (coded as '0'), or large ('1'). Size of reduced wings varied, but they were always much smaller than the elytra. Kavanaugh (1985) concluded that the loss of flying ability is biologically the most significant step in wing reduction. In light of this, the variation in amount of reduction was not explored further.

4. Secondary sclerotized island at the fold in the hind wing. Approximately two-thirds out from the wing base, the costa, subcosta, media, and radius veins of beetle wings are broken. Along these breaks, the wing folds transversely, to allow the distal part of the wing to pivot under the proximal part, and thus fit under the elytra. In this broken region is a large sclerotized patch which Ward (1979) refers to as the 'island' (I_1; Figure 2.8). This island probably aids in wing folding. Just anterior to it in some specimens is a secondary island (I_2; Figure 2.8). Specimens were coded as '0' (I_2 absent) or '1' (I_2 present). In some specimens the secondary island was deformed, very small, or attached to the primary sclerotized patch. These conditions were coded as 'd', 't', and 'a', respectively.

Whenever possible, both wings were examined, and the average condition taken.

2.3.3 Quantitative Observations

1. Body size. It was very difficult to measure total body length consistently, due to the curvature of the elytra and the varying positions of head and thorax. Therefore, length of pronotum in millimeters, measured along the midline (hereby abbreviated as PLN), was selected as a more reliable size indicator.

2. Shape of the pronotum. Although pronotal shape appeared to be highly variable within populations, differences between P. fossifrons and P. stygicus were still apparent. In a previous study (Pohl, unpublished), no significant differences were found in the taper of the pronotum (maximum width divided by posterior width).

Difference in pronotal squareness was explored in detail. A measure was desired which was not too dependent on the previously-examined PLN, yet which would clearly reflect any differences in pronotum shape. To do this, the largest samples of both macropterous and brachypterous P. fossifrons[1], and P. stygicus were selected (P. fossifrons from Rock Pond, P. fossifrons from Vancouver I., and P. stygicus from Cranbrook, respectively). For these samples, Pearson correlations were calculated separately for males and females, for PLN versus each of the following measures: pronotum width, pronotal length divided by width, and pronotal width minus length. For all species the maximum width was used as pronotal width. The 'pronotal width minus length' measure was slightly dependent on PLN (average Pearson correlation = 0.142; average n = 34), but it was much less dependent than 'pronotal width' or 'pronotal length divided by width' (average Pearson correlations of 0.740 and -0.422, respectively; average n = 34).

As well, PLN versus pronotal width was plotted for all specimens according to species (Figure 2.9). Lines were fitted through these points, and compared. This figure provides a rough indication of the relationship between pronotal length and width. P. stygicus and P. fossifrons had similar slopes, but different Y-intercepts. The slopes were almost exactly 1.0, so beetles appeared to have a predetermined pronotum shape; their pronota became equally longer and wider as they grew larger, rather than growing allometrically. Absolute squareness would not be a good measure to show any difference in Y-intercept, as the difference would become diluted in larger beetles.

Clearly, based on the Pearson correlations and on the PLN vs. pronotal width plots, pronotal width minus length (PWL), in millimeters, was the best measure of pronotum shape, so it was used in further analysis.

3. Antenna tip length. This measure was taken as the sum of the lengths of antennomeres nine, 10, and 11, measured at 50X magnification. Orientation of the antennae for measuring was as in Figure 2.10. It was thought that perhaps this measurement would be overly dependent on other measures, particularly overall beetle size. To test for this, the largest samples of macropterous and brachypterous P. fossifrons and P. stygicus were

[1] Both fully-winged and brachypterous Pfs were tested, to account for possible differences between Darlington's wing-dimorphic P. fossifrons dimorphicus and short winged P. fossifrons fossifrons subspecies.

again examined. Two standardized measures were calculated; antenna tip length divided by PLN, and antenna tip length divided by pronotal width. The antenna tip length and the two standardized measurements were then compared to PLN and PWL. Pearson correlations were calculated, to test for dependence. All three antenna measures were somewhat dependent on both pronotal measures, but the unstandardized antenna tip length was the least dependent. (average Pearson correlation = 0.445 for tip length vs. PLN; 0.202 for tip length vs. PWL; average n = 34). Therefore, unstandardized antenna tip length (ALN), in millimeters, was selected for further analysis.

4. Antennomere width. The width of the ninth antennomere was measured at 50X magnification from its widest perspective, as shown in Figure 2.10. This article was selected because it appears to be the widest segment, and because it is easy to measure. Again, the largest samples of macropterous and brachypterous P. fossifrons and P. stygicus were examined for dependence of antennomere width on other measures. For use as possible standardized measures, the ninth antennomere width was converted to a squareness measure (length divided by width), and was also divided by PLN, and by pronotal width. These three standardized measures and the unstandardized measure were correlated to PLN, to PWL, and to ALN. Of these, the ninth antennomere squareness (ASQ) was the least dependent, though it was somewhat dependent on ALN (average Pearson correlation for ASQ vs. PLN = 0.113, for ASQ vs. PWL = 0.155, and for ASQ vs. ALN = 0.371; average n = 34).

5. Length of R_1 vein of hindwing. On the leading edge of beetle hindwings, distal to the costal hinge (sensu Hammond 1979), the fused costa, subcosta, and radius form an expanded sclerotized area (SA in Figure 2.8) which helps support the distal part of the wing when it is folded under the proximal part. Apically, the sclerotized area is extended as the R_1 vein. In Patrobus, the length of this vein varied, so it was measured. To make it independent of wing size, the length of the R_1 vein was calculated as its proportion of the total length of the sclerotized area, including the R_1 vein. In Figure 2.8, the measure is $1-(a \div b)$. Whenever possible, this measure was taken on both wings, and averaged. When only one wing was available, its measure was used. This measurement is abbreviated as R1.

6. Width of the wedge cell on the hind wing. On the hind wings of many beetles, two anal veins appear to cross over and then reconnect via a cross vein. This forms the wedge cell (w in Figure 2.8), between these two anal veins. Both the length and the width of this cell vary in Patrobus. Because the two anal veins formed a very acute angle at the basal end of

the wedge cell, the length could not be measured consistently. Measuring the width was also a problem, but it was done consistently by measuring perpendicularly from the more basal, posterior anal vein, to the center of the junction of the cross-vein and the more anterior, apical anal vein (*c* in Figure 2.8). To make this measurement independent of wing size, it was divided by a standardizing measurement on a nearby vein (length of the cubital vein, between the two cubito-anal cross-veins; *d* in Figure 2.8). This standardized measurement is abbreviated as W. The cross-vein forming the apical side of the wedge cell was missing from a few specimens. Wedge cells with this condition were coded as 'open'. Another infrequent occurrence was the complete fusion of the two anal veins which form the long sides of the wedge cell. This condition was coded as 'missing'. Whenever possible, wedge cell measurements were taken on both wings, and averaged. When only one wing was available, its measurement was used.

Statistical Comparisons

These six quantitative measurements were tested for normal distribution. To do this, the largest samples of macropterous and brachypterous P. fossifrons, and P. stygicus were examined, with sexes analysed separately. Visual examination of these plots indicated that, for both males and females, the measurements approximated normal distributions. It is concluded that these measurements are in fact normally distributed in Patrobus, so they can be analyzed by statistical procedures which require such a constraint.

The following populations were sampled more than once over the three years of field collection: P. fossifrons at Pothole, and P. stygicus at Lost Rd., Chain, George, Long, and Hinton. In an attempt to rule out any temporal effects on these measurements, the different collection dates of these samples were compared via ANOVA, using the Fastat® statistics program on an Apple® Macintosh Plus® computer.

In all, 76 tests were made, to check all the samples at these sites for all six measurements. Samples of less than five beetles were excluded. At a 95% confidence interval, there was only one significant difference between samples at a site. At Chain Ponds, female P. stygicus tended to become larger over the course of the summer ($F = 4.734$; $p = 0.016$ that the samples came from the same group). This trend was not seen in males at Chain Ponds, nor was it seen at any other site. Thus it was concluded that this was due to random experimental error, which is predicted to falsely indicate significance in one out of 20 tests. It is concluded that there is no significant variation from year to year in these measurements, nor is there any significant variation through the course of a single summer. This is consistent with the conclusions based on electrophoretic data, in Section

2.2. At each of these multiply-sampled sites, the samples were pooled together for further analysis.

Males and females of the large samples used above were compared by ANOVA, using a 95% confidence interval, to test for sexual differences. Because all three populations showed significant sexual dimorphism in PWL and ALN, and at least one of the populations showed significant differences in PLN and ASQ, the sexes were kept separate for these four measurements. No sexual differences were found in the wing measurements R1 or W, so sexes were pooled for these.

2.3.4 Summary

Four qualitative measurements (microsculpture, median lobe shape, wing development, and secondary sclerotized island on the wing) and six quantitative measurements (PLN, PWL, ALN, ASQ, R1, and W) were made on P. fossifrons and P. stygicus specimens. Actual measurements (before any standardization) appear in Appendix #5. All quantitative measurements approximated normal distributions. No temporal variation within sites was found over the course of a single year, nor between different years.

2.4 Ecological Data

In southwestern Alberta, P. fossifrons and P. stygicus are generally parapatric in distribution, but there is a narrow zone of sympatry. To determine whether the two species have different habitat requirements, the ponds at which P. fossifrons and P. stygicus were collected were examined for differences. Notes were taken about the vegetation and general characteristics of all of the Patrobus sites visited by me. This information appears at appropriate points throughout the text.

2.5 Summary

Samples of the Patrobus species P. fossifrons, P. stygicus, P. lecontei, P. longicornis, P. foveocollis, P. septentrionis, and P. atrorufus, as well as the Diplous species D. aterrimus and D. californicus, were examined for eight electrophoretic loci (*Ao*, *Apk*, *Est-1*, *Est-3*, *Hk*, *Odh*, *Pgi*, and *Pgm*), for four qualitative measurements (microsculpture, median lobe shape, wing development, and secondary sclerotized island on the wing) and for six quantitative measurements (PLN, PWL, ALN, ASQ, R1, and W). Notes were also made of ecological characteristics of the collection sites.

Little significant temporal variation was found between samples collected over the course of a single summer, nor between three years' samples, so different days' samples were pooled for further analysis. These pooled samples were generally in Hardy-Weinberg equilibrium for the electrophoretic characters.

Table 2.1: Enzyme abbreviations and stain recipe references. EC numbers (in brackets) from Nomenclature Committee of the International Union of Biochemistry (1984).

ENZYME	ABBREVIATION	REFERENCE
aldehyde oxidase	AO (1.2.3.1)	Rolseth & Gooding 1978
arginine phosphokinase	APK (2.7.3.3)	Gooding & Rolseth 1979
esterases	EST (3.1.1.2)	Brewer 1970
hexokinase	HK (2.7.1.1)	Brewer 1970
octanol dehydrogenase	ODH (1.1.1.73)	Ayala *et al.* 1972
phosphoglucose isomerase	PGI (5.3.1.9)	Shaw & Prasad 1970
phosphoglucomutase	PGM (2.7.5.1)	Shaw & Prasad 1970

Table 2.2: Cases of Hardy-Weinberg disequilibrium in electrophoretic data. χ^2 = chi-squared value, **df** = degrees of freedom, and **p** = probability that the sample conforms to Hardy-Weinberg equilibrium.

SPECIES & POPULATION	LOCUS	χ^2	df	p	reason for disequilibrium
Patrobus fossifrons					
Rock	*Ao*	7.267	1	.007	homozygote of rare allele G
Rock	*Pgm*	12.025	3	.007	homozygote of rare allele I
Tern	*Pgm*			.027*	homozygote of rare allele A
Wauconda	*Odh*			.041*	deficiency of FH heterozygotes
P. stygicus					
Chain (VIII-22-89)	*Est-3*			.011*	deficiency of JL heterozygotes
George (VII-06-89)	*Pgi*	9.144	3	.027	heterozygote of rare alleles B & I
Lynch (VII-01-89)	*Est-1*			.021*	deficiency of AB & AF heterozygotes
Diplous californicus					
Similkameen	*Est-1*	5.916	1	.015	deficiency of CG heterozygotes

*indicates a Fisher exact probabilities significance test.

Table 2.3: Chi-squared-tests of electrophoretic data for heterogeneity among different days' samples. χ^2 = chi-squared value, **df** = degrees of freedom, and **p** = the probability that the samples are homogeneous.

SPECIES AND LOCALITY	χ^2	df	p
Patrobus fossifrons Lynch	8.722	5	.12070
P. stygicus Chain	31.136	22	.09337
P. stygicus George	12.734	14	.9>p>.5*
P. stygicus Hinton	19.542	10	.03390
P. stygicus Long	19.109	18	.5>p>.1*
P. stygicus Lost Rd.	9.303	12	.67683
Diplous aterrimus Rocky Mt. House	29.235	14	.00971

*Some collection dates at sites George and Long were not sampled for all loci, so tests at different loci involve different samples. Final p values are calculated manually over all loci, and compared to values in a statistical table.

Table 2.4: Pooled samples not conforming to Hardy-Weinberg equilibrium. χ^2 = chi-squared value, **df** = degrees of freedom, and **p** = the probability that the sample conforms to Hardy-Weinberg equilibrium.

POPULATION	LOCUS	χ^2	df	p	reason for disequilibrium
Patrobus stygicus					
Chain	*Est-3*	7.954	3	.047	deficiency of JL heterozygotes
George	*Pgi*	15.724	3	.001	heterozygote of rare alleles B & I
Hinton	*Pgi*	11.778	3	.008	heterozygote of rare alleles B & I
Long	*Est-1*	11.026	3	.012	deficiency of AB heterozygotes
Long	*Est-3*	12.608	3	.006	heterozygote of rare alleles G & L
Diplous aterrimus					
Rocky Mt. House	*Est-1*	9.657	3	.022	deficiency of BD heterozygotes

Table 2.5: Allele frequencies of Patrobus and Diplous population samples. Rf values are based on 7% pH 8.2 gels for *Ao*, *Apk*, *Odh*, *Pgi*, and *Pgm*; 7% pH 8.9 gels for *Est-3*; and 9% pH 8.9 gels for *Est-1* and *Hk*. See Table 2.6 for single specimen samples. Numbers in brackets refer to the number of specimens sampled for each locus.

LOCUS		SPECIES AND LOCALITIES												
		P. fossifrons					P. stygicus							
allele	Rf	Curlew	Lynch	Rock	Tern	Wauconda	Chain	George	Hinton	Long	Lost Rd.	Lynch	Pecten	Wauconda
Ao		(5)	(36)	(53)	(14)	(18)	(61)	(89)	(25)	(43)	(54)	(9)	(8)	(9)
A	.170	0	0	0	0	0	0	0	0	0	0	0	0	0
B	.225	0	0	0	0	0	0	0	0	0	0	0	0	0
C	.250	0	0	0	0	0	0	0	0	0	0	0	0	0
D	.265	0	0	0	0	0	0	0	0	0	0	0	0	0
E	.275-.310	0	0	0	0	0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
F	.315-.330	1.0	1.0	.953	1.0	0	0	0	0	0	0	0	0	0
G	.340	0	0	.047	0	1.0	0	0	0	0	0	0	0	0
Apk		(5)	(36)	(45)	(19)	(18)	(69)	(77)	(25)	(16)	(65)	(9)	(8)	(9)
A	.686	0	0	0	0	0	0	0	0	0	0	0	0	0
B	.699	0	0	0	0	0	0	0	0	0	0	0	0	0
C	.708	1.0	1.0	1.0	1.0	1.0	1.0	.994	.980	1.0	.992	1.0	1.0	1.0
D	.730	0	0	0	0	0	0	.006	.020	0	.008	0	0	0
Est-1		(5)	(33)	(53)	(19)	(18)	(71)	(61)	(25)	(28)	(65)	(9)	(8)	(9)
A	.055	0	0	0	0	0	.746	.869	.820	.875	.838	.722	.813	.889
B	.075	0	0	0	0	0	.113	.107	.100	.107	.123	.167	.188	0
C	.100	.200	.258	.170	.158	.250	0	0	0	0	0	0	0	0
D	.110	0	0	0	0	0	0	0	0	0	0	0	0	0
E	.120	.800	.742	.830	.842	.750	0	0	0	0	0	0	0	0
F	.130	0	0	0	0	0	.141	.025	.080	.018	.038	.111	0	.111
G	.140	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 2.5, continued

Est-3		(5)	(36)	(48)	(19)	(18)	(70)	(81)	(25)	(40)	(65)	(9)	(8)	(9)
A	.325	0	0	0	0	0	0	0	0	0	0	0	0	0
B	.335	0	0	0	0	0	0	0	0	0	0	0	0	0
C	.370	0	0	0	0	0	0	0	0	0	0	0	0	0
D	.385	0	0	0	0	0	0	0	0	0	0	0	0	0
E	.405	0	0	0	0	0	0	0	0	0	0	0	0	0
F	.420	0	0	0	0	0	0	0	0	0	0	0	0	0
G	.435	0	0	0	0	0	.007	.006	0	.013	.015	0	0	0
H	.445	0	0	0	0	0	0	0	0	0	0	0	0	0
I	.455	0	0	0	0	0	0	0	0	0	0	0	0	0
J	.465	0	0	0	0	0	.821	.914	.900	.913	.862	.944	.938	.500
K	.475	.700	.625	.635	.632	.556	0	0	0	0	0	0	0	0
L	.500	0	0	0	0	0	.171	.080	.100	.075	.123	.056	.063	.500
M	.505	.300	.375	.365	.368	.444	0	0	0	0	0	0	0	0
Hk		(5)	(35)	(40)	(19)	(18)	(62)	(46)	(25)	(10)	(65)	(9)	(8)	(8)
A	.410	0	0	0	0	0	0	0	0	0	0	0	0	0
B	.440	0	0	0	0	0	0	0	0	0	0	0	0	0
C	.455	0	0	0	0	0	0	0	0	0	0	0	0	0
D	.480	0	0	0	0	0	0	0	0	0	0	0	0	0
E	.495	0	0	0	0	0	0	0	0	0	0	0	0	0
F	.510	1.0	.986	1.0	1.0	1.0	.976	.978	.980	.950	.977	1.0	1.0	1.0
G	.530	0	.014	0	0	0	.024	.022	.020	.050	.023	0	0	0
H	.540	0	0	0	0	0	0	0	0	0	0	0	0	0
Odh		(5)	(36)	(53)	(19)	(18)	(71)	(85)	(25)	(34)	(65)	(9)	(8)	(9)
A	.325	0	0	0	0	0	0	0	0	0	0	0	0	0
B	.405	0	0	0	0	0	0	0	0	0	0	0	0	0
C	.440	0	0	0	0	0	.028	.012	.040	.015	0	0	0	0
D	.475	0	0	0	0	0	0	0	0	0	0	0	0	0
E	.495	0	0	0	0	0	0	0	0	.044	.023	.167	0	0
F	.515	.100	.069	.094	0	.667	0	0	0	0	0	0	0	0
G	.565	0	0	0	0	0	.972	.982	.960	.941	.977	.833	1.0	1.0
H	.580	.900	.931	.906	1.0	.333	0	0	0	0	0	0	0	0
I	.595	0	0	0	0	0	0	0	0	0	0	0	0	0
J	.635	0	0	0	0	0	0	.006	0	0	0	0	0	0
K	.660	0	0	0	0	0	0	0	0	0	0	0	0	0
L	.675	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 2.5, continued.

M	.705	0	0	0	0	0	0	0	0	0	0	0	0	0
N	.720	0	0	0	0	0	0	0	0	0	0	0	0	0
Pgi		(5)	(36)	(53)	(19)	(18)	(71)	(85)	(25)	(35)	(65)	(9)	(8)	(9)
A	.315	0	0	0	0	0	0	0	0	0	0	0	0	0
B	.350	1.0	1.0	1.0	1.0	0	.021	.012	.020	0	.008	0	.125	0
C	.365	0	0	0	0	0	0	0	0	0	0	0	0	0
D	.390	0	0	0	0	0	0	0	0	0	0	0	0	0
E	.400	0	0	0	0	.944	.972	.959	.900	.929	.985	.944	.875	.778
F	.415	0	0	0	0	0	0	0	0	0	0	0	0	0
G	.440	0	0	0	0	0	0	0	0	0	0	0	0	0
H	.460	0	0	0	0	0	0	0	0	0	0	0	0	0
I	.480	0	0	0	0	.056	.007	.029	.080	.071	.008	.056	0	.222
J	.495	0	0	0	0	0	0	0	0	0	0	0	0	0
K	.510	0	0	0	0	0	0	0	0	0	0	0	0	0
Pgm		(5)	(36)	(52)	(19)	(18)	(66)	(69)	(23)	(37)	(64)	(9)	(8)	(9)
A	.555	0	0	0	.053	0	0	0	0	0	0	0	0	0
B	.595	.100	.042	.019	0	0	.038	.029	.087	.014	.008	0	0	0
C	.615	0	0	0	0	0	0	0	0	0	0	0	0	0
D	.635	.900	.958	.942	.947	.389	.939	.913	.870	.905	.961	1.0	.813	.944
E	.650	0	0	0	0	0	0	0	0	0	0	0	0	0
F	.660	0	0	0	0	0	0	0	0	0	0	0	0	0
G	.670	0	0	0	0	0	.008	.022	.043	.068	.016	0	.188	0
H	.680	0	0	0	0	0	0	0	0	0	0	0	0	0
I	.690	0	0	.038	0	.556	.015	.036	0	.014	.016	0	0	.056
J	.700	0	0	0	0	0	0	0	0	0	0	0	0	0
K	.720	0	0	0	0	.056	0	0	0	0	0	0	0	0

Table 2.5, continued.

LOCUS allele	Rf	SPECIES AND LOCALITIES							
		P. lecontei		P. longicornis	P. atrorufus	P. septentrionis	D. aterrimus		D. californicus
		Lynch	Rock	Edmonton	Finland	Hinton	Rocky Mt. House	Waterton	Similkameen
Ao		(9)	(3)	(9)	(9)	(6)	(34)	(5)	(23)
A	.170	0	0	0	0	0	0	0	.022
B	.225	0	0	0	0	0	.176	.100	.978
C	.250	0	0	0	0	0	.529	.800	0
D	.265	0	0	0	0	0	.294	.100	0
E	.275-.310	0	0	0	0	1.0	0	0	0
F	.315-.330	1.0	1.0	1.0	1.0	0	0	0	0
G	.340	0	0	0	0	0	0	0	0
Apk		(9)	(3)	(9)	(9)	(6)	(42)	(6)	(33)
A	.686	0	0	0	0	0	1.0	1.0	1.0
B	.699	0	0	0	0	0	0	0	0
C	.708	.944	1.0	1.0	1.0	1.0	0	0	0
D	.730	.056	0	0	0	0	0	0	0
Est-1		(6)	(3)	(9)	(3)	(6)	(38)	(6)	(24)
A	.055	.167	.167	0	0	.167	0	0	0
B	.075	0	0	0	0	0	.224	0	0
C	.100	.167	.833	.889	.833	0	0	0	.458
D	.110	0	0	0	0	0	.671	.917	0
E	.120	0	0	.111	.167	.250	0	0	0
F	.130	.667	0	0	0	.583	0	0	0
G	.140	0	0	0	0	0	.105	.083	.542

Table 2.5, continued.

Est-3		(9)	(3)	(9)	(9)	(6)	(48)	(6)	(33)
A	.325	.111	0	0	0	0	0	0	0
B	.335	.889	1.0	0	0	1.0	0	0	0
C	.370	0	0	0	.389	0	0	0	0
D	.385	0	0	0	0	0	0	0	0
E	.405	0	0	.389	.278	0	.094	.083	0
F	.420	0	0	0	0	0	0	0	0
G	.435	0	0	.500	.333	0	0	0	.773
H	.445	0	0	0	0	0	0	0	0
I	.455	0	0	0	0	0	.906	.917	.227
J	.465	0	0	.111	0	0	0	0	0
K	.475	0	0	0	0	0	0	0	0
L	.500	0	0	0	0	0	0	0	0
M	.505	0	0	0	0	0	0	0	0
Hk		(8)	(3)	(9)	(9)	(6)	(22)	(6)	(33)
A	.410	0	0	0	0	0	0	0	.061
B	.440	0	0	0	0	0	.977	1.0	.939
C	.455	0	0	0	0	0	.023	0	0
D	.480	0	0	0	.111	0	0	0	0
E	.495	0	0	1.0	0	0	0	0	0
F	.510	0	0	0	.889	1.0	0	0	0
G	.530	.938	1.0	0	0	0	0	0	0
H	.540	.063	0	0	0	0	0	0	0
Odh		(9)	(3)	(9)	(9)	(6)	(48)	(5)	(33)
A	.325	0	0	.667	0	0	0	0	0
B	.405	0	.167	0	.778	0	0	0	0
C	.440	0	0	0	0	0	0	0	0
D	.475	0	0	.278	0	0	0	0	0
E	.495	0	0	.056	0	0	0	0	0
F	.515	0	0	0	0	0	0	0	0
G	.565	0	0	0	0	0	0	0	0
H	.580	1.0	.833	0	.111	1.0	0	0	0
I	.595	0	0	0	.056	0	.010	0	0
J	.635	0	0	0	0	0	0	0	0
K	.660	0	0	0	.056	0	.917	.400	.030
L	.675	0	0	0	0	0	.031	0	0

Table 2.5, continued.

M	.705	0	0	0	0	0	.042	.600	0
N	.720	0	0	0	0	0	0	0	.970
Pgi		(9)	(3)	(9)	(9)	(5)	(47)	(5)	(33)
A	.315	0	0	.222	0	0	0	0	0
B	.350	0	0	0	.056	0	.011	0	0
C	.365	.944	1.0	.722	0	0	0	0	0
D	.390	0	0	0	0	.700	0	0	0
E	.400	.056	0	.056	.056	0	0	0	0
F	.415	0	0	0	0	0	.191	.300	0
G	.440	0	0	0	.278	.300	0	0	0
H	.460	0	0	0	0	0	.787	.700	.727
I	.480	0	0	0	.444	0	0	0	0
J	.495	0	0	0	0	0	.011	0	.273
K	.510	0	0	0	.167	0	0	0	0
Pgm		(9)	(2)	(9)	(9)	(4)	(39)	(5)	(33)
A	.555	0	0	0	0	0	0	0	0
B	.595	0	0	0	0	0	0	0	.061
C	.615	0	0	0	0	1.0	0	0	.742
D	.635	0	0	0	.111	0	0	0	0
E	.650	0	0	0	0	0	.436	.400	0
F	.660	1.0	1.0	.833	.056	0	0	0	.197
G	.670	0	0	0	.833	0	0	0	0
H	.680	0	0	0	0	0	.269	.300	0
I	.690	0	0	.167	0	0	0	0	0
J	.700	0	0	0	0	0	.295	.300	0
K	.720	0	0	0	0	0	0	0	0

Table 2.6: Genotypes of single-specimen samples of Patrobus and Diplous. Refer to Table 2.5 for Rf values of alleles.

Species/Population	*Ao*	*Apk*	*Est-1*	*Est-3*	*Hk*	*Odh*	*Pgi*	*Pgm*
P. fossifrons								
Deep	G G	C C	E E	K K	F F	F H	B E	D I
Manning	G G	B B	E E	I K	F F	H H	B B	I I
Pothole	F F	C C	C E	K M	F F	H H	B B	D D
Train	G G	C C	C C	L L	F F	F H	E E	D I
P. stygicus								
Antenna	E E	C C	A A	J L	F F	G G	E E	D D
Blakiston	E E	C C	A A	J L	F F	G G	E E	D D
P. fossifrons/ stygicus hybrid								
Wauconda	E G	C C	F F	K L	F F	F F	E E	I I
P. lecontei								
Bennett	F F	C C	A A	B B	G G	H H	C E	F F
Curlew	F F	C C	A A	B B	G G	H H	C E	F F
George	F F	C C	A A	B B	G G	H H	C C	F F
Pecten	F F	C C	A C	B B	G G	H H	C C	F F
Tern	F F	C C	F F	B B	G G	H H	C C	F F
P. foveocollis								
Hinton	E E	D D	F F	B B	G G	C C	B B	A A
Long	E E	C C	- -	B B	G G	C D	B B	A A
D. aterrimus								
Oldman	C D	A A	D D	D H	B B	K K	H H	E H

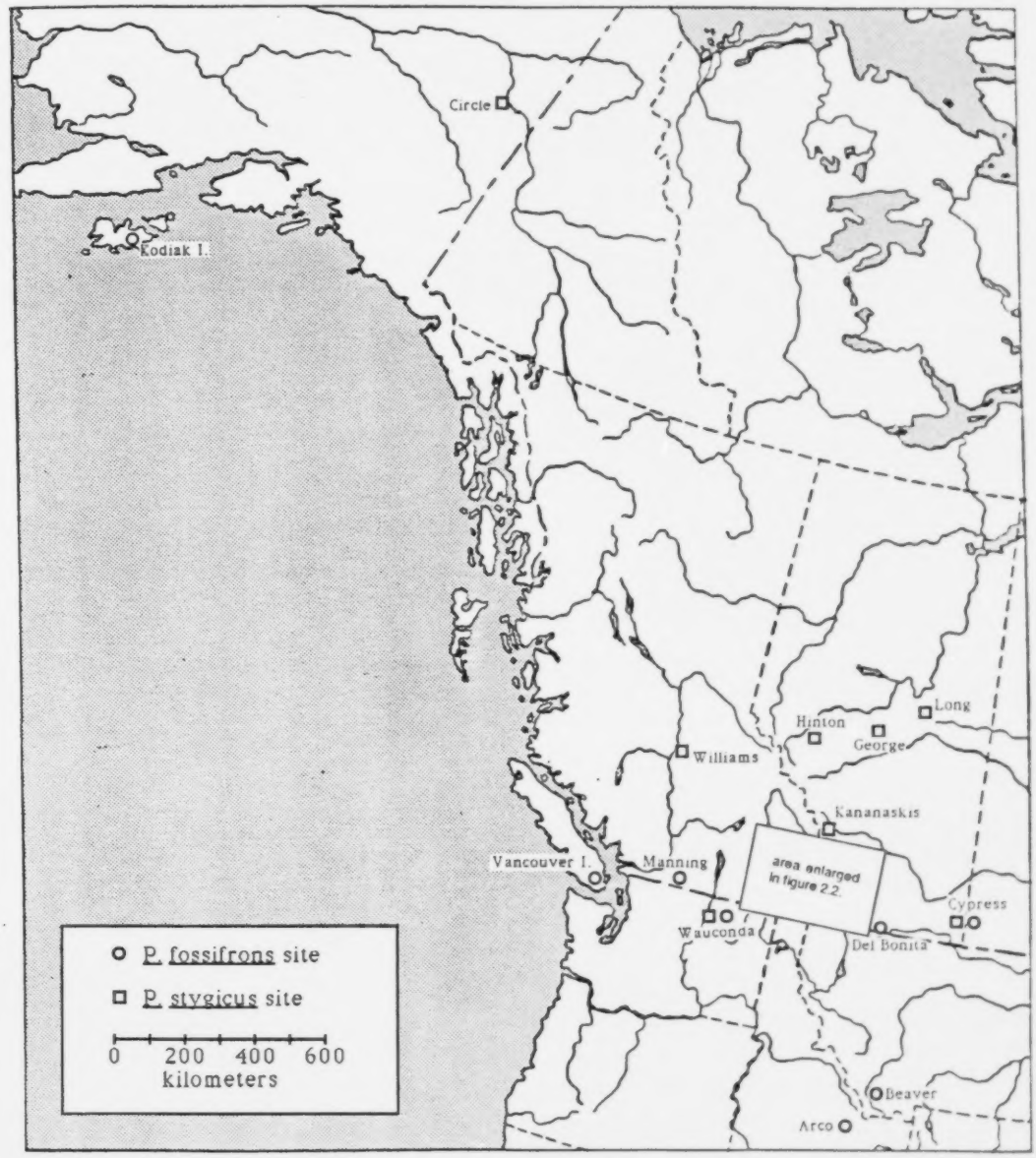


Figure 2.1: Collection sites of Patrobus fossifrons and P. stygicus in western North America.

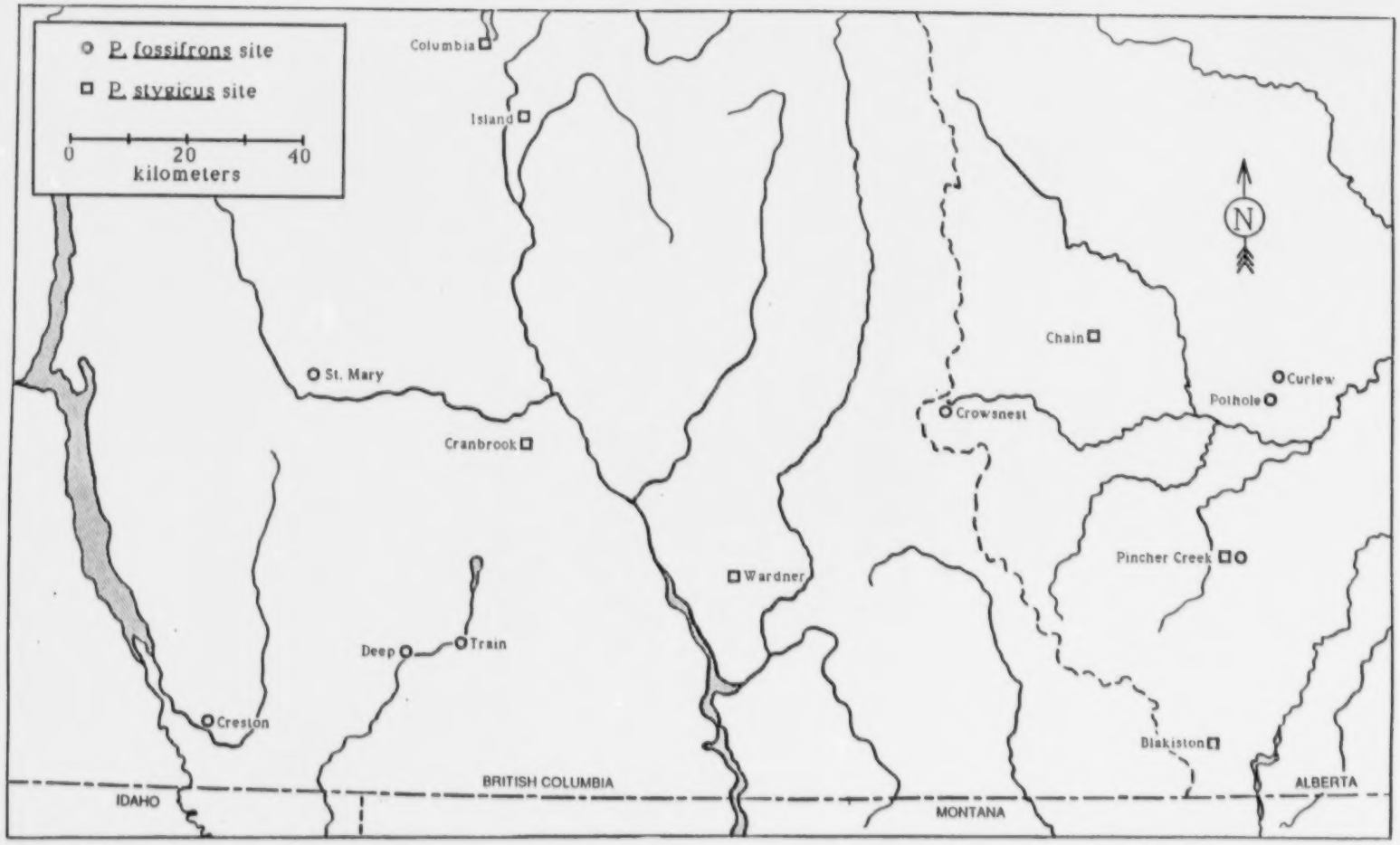


Figure 2.2: Collection sites of Patrobus fossifrons and P. stygicus in southeastern British Columbia and southwestern Alberta.

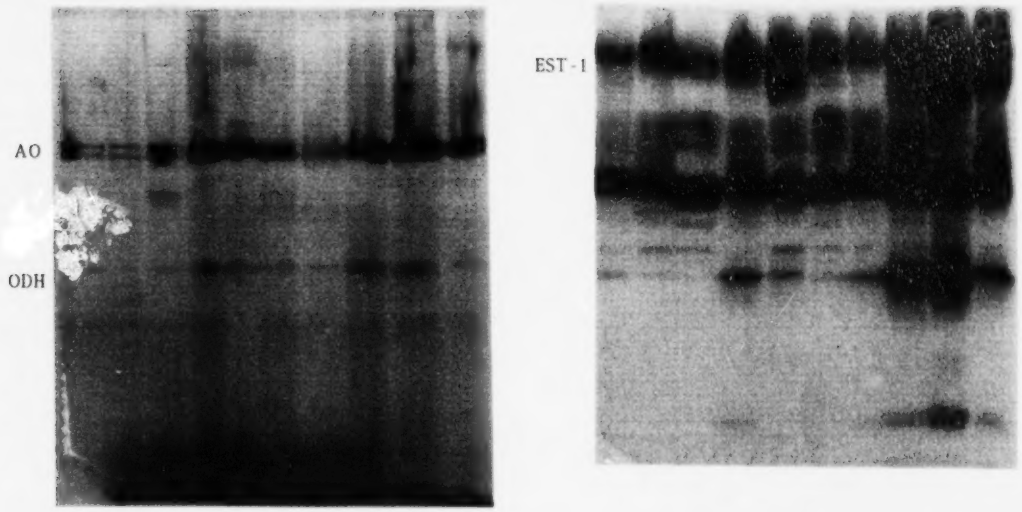


Figure 2.3: Examples of electrophoretic gels stained for AO, ODH, and EST. AO and ODH bands are on a 7% pH 8.2 gel, and EST bands are on a 9% pH 8.9 gel. Migration was from top (cathode) to bottom (anode), from 10 slots containing Patrobus stygicus homogenates. Beetle genotypes were interpreted as follows: *Ao* - all EE; *Odh* (left to right) - GG, GJ, GG, GG, GG, GG, GG, GG, GG, GG; *Est-1* (left to right) - AA, AA, AB, AA, AF, AA, AA, AB, AB, AF.

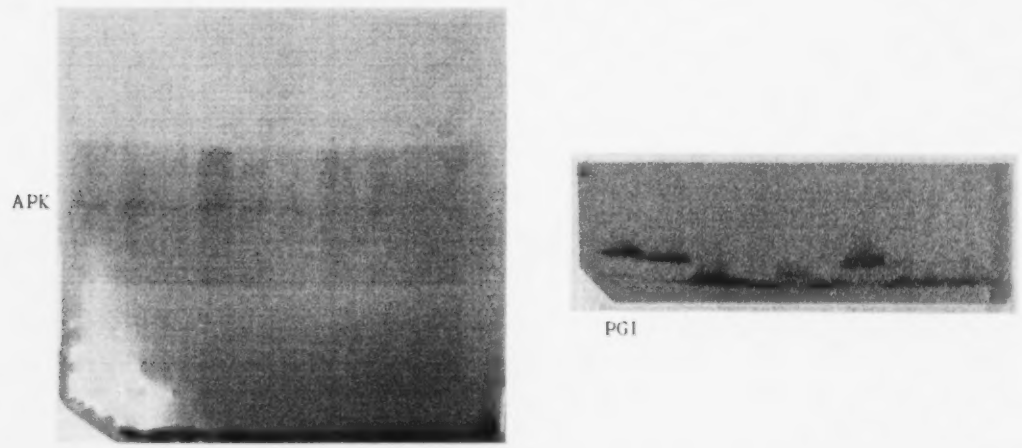


Figure 2.4: Examples of electrophoretic gels stained for APK and PGI. APK bands are on a 9% pH 8.9 gel, and PGI bands are on cellulose acetate overlaid on a 7% pH 8.2 gel. Migration was from top (cathode) to bottom (anode), with seven slots on the left containing Patrobus fossifrons, and three slots on the right containing P. stygicus homogenate. Genotypes were interpreted as follows, from left to right: *Apk* - CC, CC, CC, CC, CC, CC, BB, CC, CC, CC; *Pgi* - BB, BB, EE, EE, BE, EE, BB, EE, EE, EE.

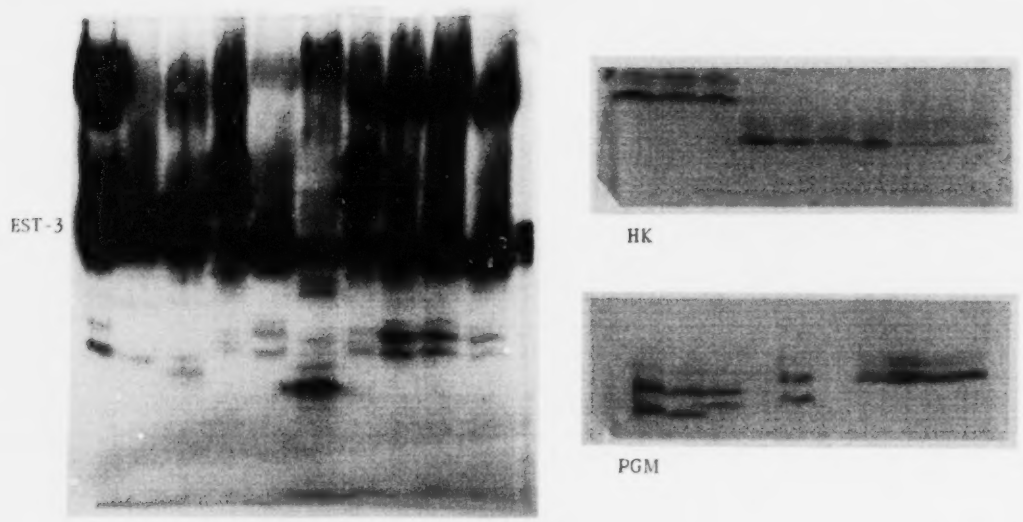


Figure 2.5: Examples of electrophoretic gels stained for EST, HK, and PGM. EST bands are on a 7% pH 8.9 gel, and HK and PGM bands are on cellulose acetate overlays, from a 9% pH 8.9 gel, and a 7% pH 8.2 gel, repectively. Migration was from top (cathode) to bottom (anode). From left to right, sample slots contained Diplous aterrimus (slots 1-3); Patrobus septentrionis (slot 4); P. stygicus (slots 5-6); P. septentrionis (slot 7); and P. fossifrons (slots 8-10). Genotypes were interpreted as follows, from left to right: *Est-3* - HH, HH, DH, BB, JJ, JJ, BB, KK, KK KM; *Hk* - BB, BB, BB, FF, FF, FF, FF, FF, FF, FF; *Pgm* - EJ, EJ, EH, CC, EG, unknown, CC, EE, EE, EE.

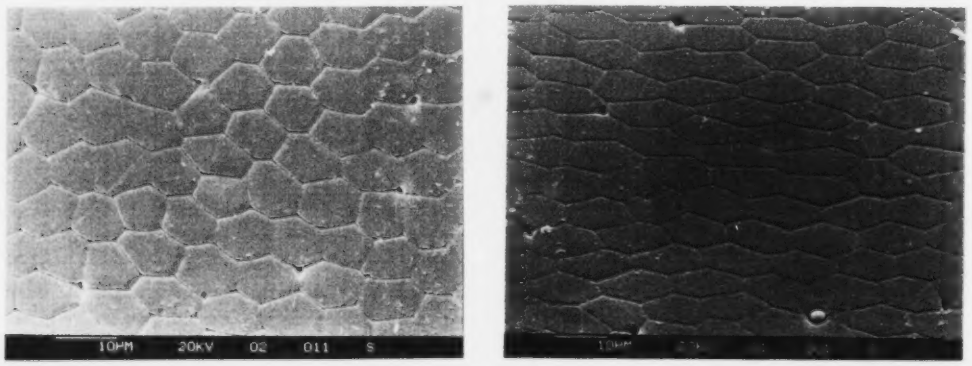


Figure 2.6: Elytral microsculpture micrographs. Left: isodiametric microsculpture of Patrobus fossifrons (character state '1'); right: transverse microsculpture of P. stygicus (character state '5').

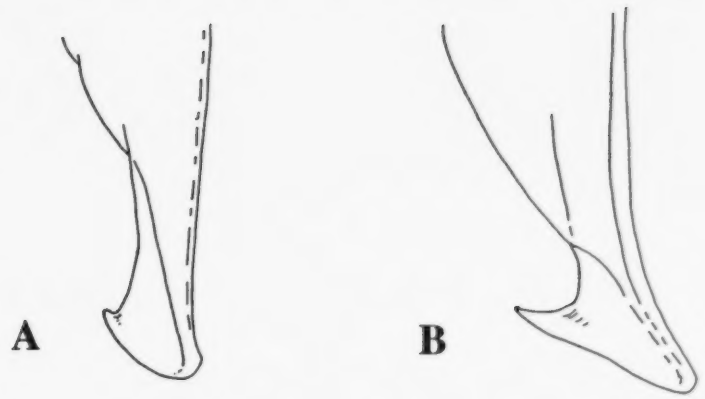


Figure 2.7: Penis apices (right side view) of; A, Patrobus fossifrons (character state '1'), and B, P. stygicus (character state '5'). Approximately 30X magnification. Based on figures in Lindroth (1961).

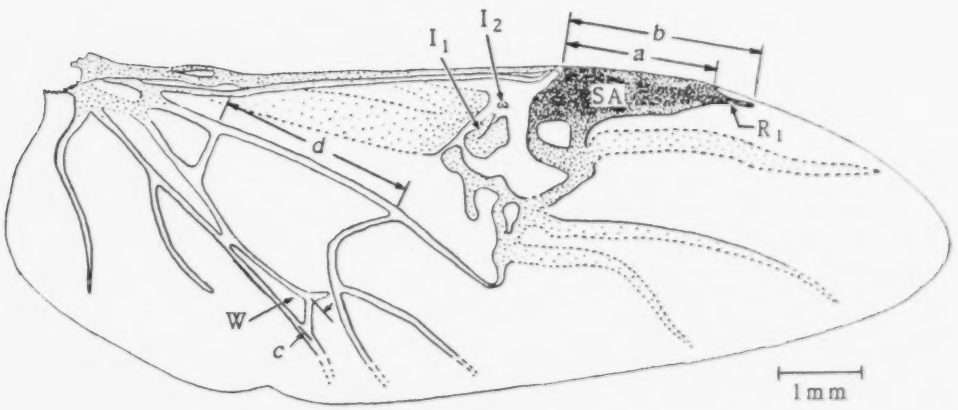


Figure 2.8: Wing of Patrobus fossifrons, showing measurements taken. I_1 = primary sclerotized island; I_2 = secondary sclerotized island; SA = sclerotized area on fused costa, subcosta, and radius; R_1 = first branch of radial vein; *a* and *b* = measurements made to calculate the relative length of R_1; W = wedge cell; and *c* and *d* are measurements made to calculate the relative width of W.

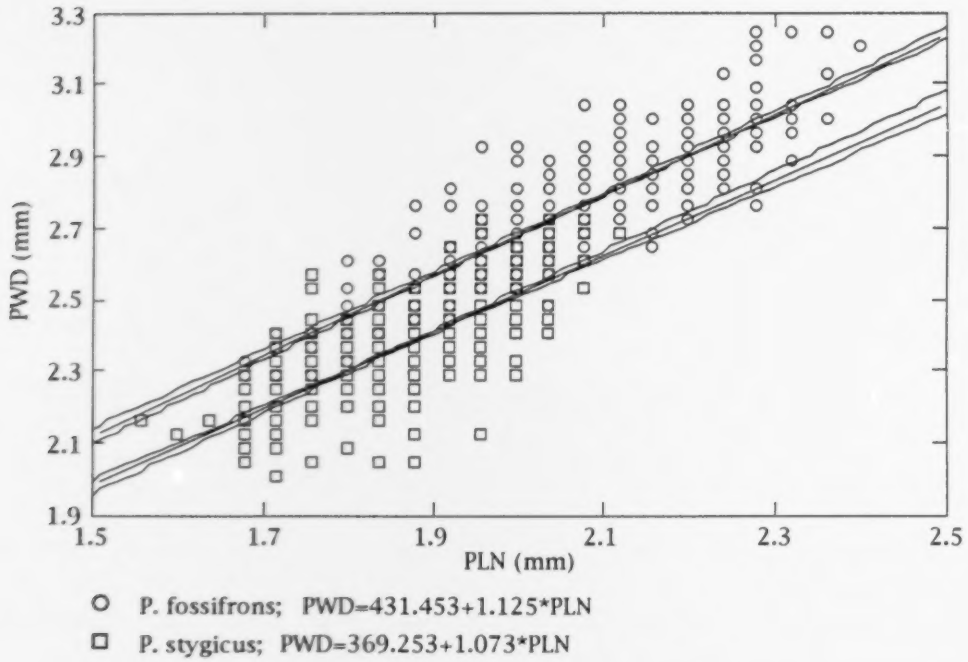


Figure 2.9: Pronotal length (PLN) versus pronotal width (PWD) for all Patrobus fossifrons and P. stygicus specimens. Error curves on lines represent 95% confidence intervals.

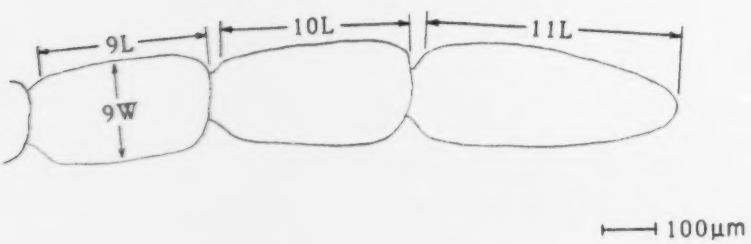


Figure 2.10: Tip of antenna of Patrobus fossifrons, anterior aspect, showing measurements taken. 9L, 10L, and 11L = lengths of antennomeres nine, 10, and 11; 9W = width of antennomere nine.

3. EVALUATION OF SPECIES AND MORPHS

3.1 Introduction

In preparation for this study, samples of P. fossifrons (Pfs) and P. stygicus (Pst) were obtained from 37 sites. Most of these sites were occupied by only one of the two species. However, at Cypress Hills, Alberta, Pincher Creek, Alberta, and Wauconda, Washington, both species were found. To examine these sites of sympatric occurrence, the characteristics of Pfs and Pst in allopatry must be known. To obtain this information, the sites where Pfs or Pst occur in allopatry are analyzed and compared here.

3.2 Data Examined

Population samples were examined for qualitative and quantitative morphological characters, and electrophoretic and ecological characters. All allopatric samples were collected at least 25km from any known record of the other species, and were distributed from Alaska, south and east through British Columbia and Alberta, into Washington, Idaho, and Montana. They have been arranged in a generally northwest to southeast order in the following tables and figures.

Table 3.1 lists qualitative morphological characteristics. Character states were not constant within populations, so the average character state for each population has been calculated here. For character I_2, individuals with tiny, deformed, or attached secondary sclerotized islands were considered to be intermediate between conditions '0' and '1' for these calculations. Refer to Section 2.3.2 for details of the measurement of this and other characters.

The quantitative characters, depicted as diagrams based on the method of Hubbs and Hubbs (1953), appear in Figures 3.1 to 3.10. In these plots, each character is depicted by a series of lines and boxes. The horizontal line indicates the range of measurements, and the vertical line indicates the mean. For samples of ten or more specimens, an outlined box indicates 1.5 standard deviations to each side of the mean, and a shaded box indicates two standard errors to either side of the mean. The number of individuals in each sample is indicated in brackets. The standard error boxes measure the certainty of means. According to Hubbs and Hubbs, if these boxes do not overlap, or overlap only slightly, then a biologically-significant difference exists between the populations being compared. This is the criterion used in this study to determine significance of differences between populations. The standard deviation boxes measure the dispersion about the mean, and are

not affected by sample size, except in very small samples. If these boxes do not overlap, then there is at least 93% separation between populations. This is generally thought to approximate the level of difference between subspecies (Mayr 1969; p. 189-193), though a standard level of difference between subspecies is not universally agreed upon.

Some population samples were analyzed for electrophoretic characters as well. Electrophoretic data appear in Tables 2.5 and 2.6. For samples with at least five individuals, Nei's genetic identity measures have been calculated between all possible pairs of populations (Table 3.2). Nei's (1972) original identity measure is biased with small sample sizes, so Nei's (1978) unbiased identities, which correct for small sample size, are used here:

[3.1] $$D = -\ln\left[\frac{J_{XY}}{\sqrt{J_X J_Y}}\right]$$

where **D** = Nei's unbiased distance, and $\mathbf{J_{XY}}$, $\mathbf{J_X}$, and $\mathbf{J_Y}$ are the means over all loci of $\Sigma X_i Y_i$, $(2N_x\Sigma X_i^2-1)/(2N_x-1)$, and $(2N_y\Sigma Y_i^2-1)/(2N_y-1)$, respectively ($N_x$ and N_y are the sample sizes, and X_i and Y_i are the frequencies of alleles i, in populations X and Y). A more detailed theoretical discussion of this and other distance measures appears in Chapter 6.

3.3 Results

3.3.1 Character States of Allopatric Stocks of P. stygicus

Qualitative Morphological Characters

All of the allopatric Pst populations had at least partly transverse microsculpture, and males had markedly fish-hook shaped median lobes. Samples examined for wing characters were entirely macropterous, and had a very low presence of the secondary sclerotized island.

Quantitative Morphological Characters

Pst pronota were highly consistent for both length (PLN; Figures 3.1 and 3.2) and width minus length (PWL; Figures 3.3 and 3.4). No single population was significantly different from all the others. For antennal segment length (ALN; Figures 3.5 and 3.6), all the populations were very similar, except for Circle, Alaska, for which the males and females had significantly smaller values. This is not due to overall body size, since they

were not the smallest beetles. Regarding antennal segment squareness (ASQ; Figures 3.7 and 3.8), the northern Circle and Williams Lake populations were the least elongate, while the central Albertan sites at Long Lake, George Lake, and Hinton were the most elongate. The relative length of the R_1 wing vein (R1; Figure 3.9) and the relative width of the wedge cell (W; Figure 3.10) both tended to decrease with latitude. Though the latter two characters were quite variable over the entire geographical range of Pst sites, at individual sites they were more constant. As will be seen in the next chapter, they can be useful to identify populations of Pst.

Overall, the allopatric Pst were very similar morphologically, except for slight differences in ALN and ASQ.

Electrophoretic Characters

Electrophoretic data also show that allopatric Pst are very closely related; Nei's unbiased genetic identities for the four electrophoresed samples ranged from 0.997 to 1.0 (Table 3.2).

Ecological Characteristics

Little is known of the collection sites of the pinned Pst specimens collected prior to 1980. The specimens from Circle, Alaska bear the label "Carex marsh". Today, Columbia Lake is a large open body of water, but the south end, where these specimens were collected, has extensive areas of still water and large patches of Scirpus and Typha. Island Pond, between Kimberley and Canal Flats, persists as a woodland pond, but it is currently used by cattle as a watering hole. No vegetation is present around its perimeter, and few carabids live at its edge. (Incidentally, cattle were the major hindrance to collecting samples during this study. Heavy cattle use devastates the habitat of Patrobus and many other carabids, and poses a serious threat to their survival.)

Long Lake pond is a permanent Carex/Salix marsh in a mixed aspen/spruce forest. George Lake is a large body of water in aspen parkland, with extensive Carex/Salix patches at the west end. Hinton Pond is a small, permanent Carex/moss pond in spruce forest. Chain Ponds is a string of beaverponds at 1480m in the Livingstone Range. It is a Carex/Salix marsh in willow/aspen parkland. The Blakiston site is an oxbow on Blakiston Creek, in Waterton Park. It is a clear, cold trout stream in a mixed aspen/boreal forest. The oxbow is full of Carex and Salix. Nothing is known of the ecological characteristics of the remaining allopatric Pst sites.

3.3.2 Character States of Allopatric Stocks of P. fossifrons

Qualitative Morphological Characters

Except for the individual collected at Deep Pond, all the Pfs had completely or almost completely isodiametric microsculpture (Table 3.1). All males had median lobes that were spoon-shaped, with very small barbs. Hindwing development and presence of the sclerotized island on the wing varied.

Quantitative Morphological Characters

All allopatric Pfs had similar measurements for PWL (Figures 3.3 and 3.4), and W (Figure 3.10). R1 (Figure 3.9) was also consistent, except for the Manning and Creston specimens.

In females, the characters PLN (Figure 3.1) and ALN (Figure 3.5) grouped the Kodiak Island, Vancouver Island, Manning, St. Mary, and Crowsnest populations as significantly different from the Curlew, Pothole, Del Bonita, Beaver, and Arco populations. In males, this trend distinguishing more northwestern specimens from more southeastern ones was less obvious; the Train specimen was intermediate between the two groups for PLN (Figure 3.2), and the Beaver and Arco specimens were intermediate for ALN (Figure 3.6). For ASQ (Figures 3.7 and 3.8), more northwestern beetles were again different from more southeastern ones, with Train, Beaver, and Arco specimens somewhat intermediate.

Electrophoretic Characters

Electrophoretic data are available for only one allopatric Pfs sample having at least five individuals. Pairwise distance and identity measures thus could not be calculated. However, the allele frequencies of this and the smaller samples (Tables 2.5 and 2.6) provide some idea of the relationships within the taxon.

The fixed difference at the *Ao* locus groups Deep, Train, and Manning as distinct from Pothole and Curlew. The Manning beetle had unique alleles at *Apk* and *Est-3*, setting it apart from Deep and Train. The Train individual was fixed for allele L at *Est-3*, indicating that it is somewhat isolated from other Pfs as well. At the other loci, there were no clear differences, due largely to the small amount of data collected from Pfs west of the Rocky Mountains.

Ecological Characteristics

Although some of the museum specimens of Pfs were from creeks or unknown types of habitat, all the Pfs that I collected were from pond margins. This does not agree with

Darlington (1938). He found Pfs to be less hygrophilous, and to be associated with rivers rather than ponds. During my collecting, I visited both types of sites but I found Pfs at marshes only. This may be an artifact of the types of sites Darlington collected, or perhaps Pfs prefers river habitats in the Pacific Northwest, where he did much of his collecting (and where, incidentally, I found very few Pfs).

The Kodiak Island population sample includes specimens collected between 1958 and 1962 at several marshes, creeks, and meadows. The Vancouver Island specimens came from Elk Lake and Beaver Lake (two attached lakes near Victoria; collected in 1955), and from Duncan (collected in 1958). I found no beetles when I visited the area in 1989. At that time, the Elk Lake and Beaver Lake sites contained very coarse sedge clumps, quite unlike mainland Patrobus sites. Many ponds of this nature also existed in the Duncan area in 1989, none of which yielded beetles then. The Manning site, where only one beetle was found, was a series of marshy areas along a mountain stream. The Carex there was very tall and unclumped. The Creston specimen was collected in 1958. In 1988 and 1989 there were extensive marshy areas around Creston containing very tall Carex, both in clumps and evenly distributed. No beetles were found there in those years. Deep Pond also contained tall Carex, in deep water. The nearby Train Pond had much finer Carex, unclumped in a wet meadow. The St. Mary site (St. Mary's Lake) is a widening of the St. Mary River. It has sedge fields at the west end, but clumps have not formed because the water level is highly unstable. The specimens examined here were collected in 1955. No beetles were found there in 1988 or 1989. The Crowsnest site is a widening of the Crowsnest River near Crowsnest Lake, just east of the continental divide. The Crowsnest specimens were collected in 1955 and 1956. Today it has extensive Carex clumps and Typha. No beetles were found there in 1986 through 1989. Curlew and Pothole are dammed prairie gulches on the southeast slopes of the Porcupine Hills. There are no large trees at these sites. Both have sedge clumps and pondweed mats along their margins. The Del Bonita specimen is labelled "margin of prairie slough". The town of Del Bonita is on the treeless prairie, so there were probably no trees at this site. Nothing is known of the Beaver and Arco sites.

3.4 Discussion

3.4.1 P. fossifrons Populations

There are clearly differences between some of the Pfs populations, although different characters suggest slightly different groupings. The morphological characters PLN, ALN, and ASQ suggest that the Kodiak Island, Vancouver Island, Manning, St. Mary, and

Crowsnest populations be grouped together, and that the Curlew, Pothole, and Del Bonita populations be grouped together. The Train, Beaver, and Arco populations are somewhere in between these groups. The electrophoretic data suggest that Curlew and Pothole are somewhat distinct from Manning, Deep, and Train. Specimens from the latter three sites may be electrophoretically distinct from each other as well.

Ecological data are sketchy, but one trend is that the more western ponds have coarser sedge. If this indicates a habitat difference which somehow divides Pfs into two morphs, then the boundary between the groups would extend from the northwest to the southeast, between Deep Pond and Train Pond. However, much of these data could well be inaccurate, since they are based on descriptions of ponds at which Pfs has not been found for many years. The very lack of current populations at some of these sites indicates that the habitats are unsuitable today. Perhaps ecological shifts which allowed the coarse sedge to move in also forced Pfs into local extinction. For this reason, little weight can be placed on this ecological information.

A clearer ecological trend is that northwestern sites are in damp boreal forests, while those in the southeast are in drier parkland and prairie habitats. These characteristics are much more stable than such things as the sedge species present, so they are unlikely to have changed in the past 50 years. Therefore, this is a trend which is more certain than the sedge coarseness. In general it supports the groupings indicated by morphological and electrophoretic data; the northwest populations live in boreal habitat, while those in the south and east live in parkland and prairie. This suggests a division grouping Kodiak Island, Vancouver Island, Manning, Creston, St. Mary, and Crowsnest versus Curlew and Pothole, based on the general forest vegetation or lack thereof.

'Inland' and 'Coastal' Morphs of P. fossifrons

When all characters are taken into consideration, it is clear that Curlew, Pothole, Del Bonita, Beaver, and Arco are distinct from the others. Together, these are the 'inland' morph of Pfs (hereby abbreviated as Pfs-i). The rest of the Pfs populations comprise the 'coastal' morph of Pfs (Pfs-c). As summarized in Table 3.3, Pfs-i have longer pronota, longer antennal tips, and relatively more elongate antennal segments than Pfs-c. The latter two differences are illustrated in Figure 3.12. Pfs-i are also fixed for *Ao* allele F rather than G, predominant in *Est-3* alleles K and M rather than I, K, and L, and predominant in *Pgm* allele D rather than I.

The Beaver and Arco populations, though closer to Pfs-i, exhibit some Pfs-c characteristics (lower incidence of macroptery, lower presence of the secondary sclerotized island, and intermediate measurements for ALN and ASQ). These populations may be

mixed stocks of Pfs-c and Pfs-i, due to possible gene flow in the Idaho-Montana area. However, without electrophoretic or genetic analysis, this cannot be tested, so they are considered to be Pfs-i, to which they are closer morphologically.

The Train specimen, though closer to Pfs-c than Pfs-i, also exhibits some characteristics of both morphs. Its measurements for PLN and ALN are closer to states of Pfs-i than Pfs-c, and it is homozygous for *Est-1* allele C, which was not seen in the other two electrophoresed specimens of Pfs-c, but which was seen in Pfs-i. This suggests that gene flow between Pfs-i and Pfs-c may also be occurring in south-central British Columbia.

The electrophoretic data suggest that the Manning, Deep, and Train specimens are somewhat distinct from each other. This may be an artifact of the small sample sizes, in conjunction with the gene flow suggested above, or it may indicate that Pfs-c is further subdivided. A subdivision in the area of Deep and Train Ponds is supported by ecological data: Vancouver Island, Manning, Creston, and Deep sites appear to contain coarse sedge, while Train, St. Mary, and Crowsnest are sites with finer sedge. Until electrophoretic data can be gathered from more specimens to fully explore this possible subdivision, it will not be recognized. These populations are clearly closer to each other than to Pfs-i, so at this point they are considered to be Pfs-c.

Comparison of Pfs-i and Pfs-c to Darlington's subspecies of P. fossifrons

Because the division between Pfs-i and Pfs-c is unclear, and may in fact be permeable, these morphs are not recognized taxonomically as subspecies. They do not correspond exactly to Darlington's (1938) P. fossifrons dimorphicus and P. fossifrons fossifrons. His P. fossifrons fossifrons (type locality Unalaska Island) referred only to specimens from the Bering Sea islands and Kodiak Island, while P. fossifrons dimorphicus (type locality near Victoria, B.C.) referred to all others now known as the species Pfs. In other work, Darlington (1936) found that brachyptery was correlated with stable habitats, and that macroptery was correlated with temporary habitats. Therefore, he thought that in Pfs these different wing states were indicative of ecological differences. However, he found no other concrete differences between P. fossifrons dimorphicus and P. fossifrons fossifrons, so his division was based entirely on the wing character. Since brachyptery has been shown to be phenotypically dominant over macroptery in carabids (Lindroth 1946, Den Boer *et al.* 1980), it is possible for brachypterous carabid populations to have small amounts of macropterous genes which are not expressed. Thus the difference between brachypterous and dimorphic populations could be merely a difference in the frequency of alleles at a single gene. This is not enough of a difference to describe separate subspecies.

In contrast, I have split Pfs populations much farther south and east, grouping all British Columbia populations, except those from the extreme southeast, with Darlington's P. fossifrons fossifrons. Even if the subdivision of my Pfs-c could be specified, the groupings would not correspond to Darlington's subspecies. I have clearly shown that the Vancouver Island population, and probably some mainland populations, are similar to the Kodiak Island population. Darlington's P. fossifrons dimorphicus is consubspecific with Pfs-c, since its type locality is within the range of Pfs-c. My groupings are more reliable, since they are based on several morphological and electrophoretic characters, not just on one trait.

Pfs has a 'coast-centered' distribution, which is seen in other insects as well (Kavanaugh 1988). The range of Pfs-c is along the Pacific coast, from Alaska to California, and extends inland in southern Canada as far as the Crowsnest Pass. Pfs-i lives in south-central British Columbia, Idaho, Montana, and southern Alberta, ranging as far east as the Cypress Hills.

Biogeographical Analysis of P. fossifrons Morphs

These different morphs are clearly descendants of some past isolation event. I estimate a time of divergence at one to three million years ago, based on the age of other carabid taxa, and on the degree of electrophoretic differences between morphs (see Chapter 6 for details). Within this time frame, the Nebraskan glaciation, the first glaciation of the Quaternary Period, is the most likely event to have originally isolated the inland and coastal morphs of Pfs. This would put the date of divergence at about 1.5 million years ago. When the ice sheet advanced, the original range of Pfs was broken, and populations survived in refugia at the edges of the ice. During the thousands of years that the survivors were separated, they diverged both genetically and morphologically, to become the separate morphs of today.

It is not known where these original Pfs-i and Pfs-c refugia were located, because current distributions reflect only the most recent glaciation, the Wisconsinian, which ended 10,000 years ago. Locations of refugia from this later glaciation can be inferred from wing length information. Lindroth (1979) proposed that, in wing-dimorphic species, brachypterous populations represent old centers of distribution. Since macropterous individuals would do most of the colonizing from these old sites, newer sites would have higher levels of macroptery. Therefore, probable routes of dispersal from refugia can be traced in the direction of increasing macroptery.

Pfs-i shows a clear trend of increasing macroptery in more northern populations, with complete macroptery north of 49° latitude. The Cypress Hills specimens are completely

macropterous, whereas the Beaver and Arco populations are partly brachypterous. Thus Pfs-i appears not to have taken refuge in the Cypress Hills, which are thought to have remained ice-free during the last glaciation (Breitung 1954, Campbell and Peck 1990). Instead, all Pfs-i beetles appear to have descended from a population south of the ice sheets, perhaps in the vicinity of the Beaver (Montana) and Arco (Idaho) populations.

Kodiak Island has also been explored as a likely ice-free refuge during the last glaciation (Karlstrom and Ball, *editors*, 1969). Possibly ancestors of present-day Pfs-c migrated south along the coastal islands to Vancouver Island, and then migrated inland to occupy present-day sites. This hypothesis is supported by levels of brachyptery; Kodiak Island specimens were all brachypterous, the Vancouver Island sample contained 4.9% macropterous beetles, and the Manning and Creston specimens were macropterous.

The origin of Pfs-c in eastern British Columbia is somewhat contentious. If beetles in this area are descended from Kodiak Island refugium stock, they ought to have high levels of macroptery, like the Manning and Creston specimens. However, Deep, Train, St. Mary, and Crowsnest beetles are all brachypterous. This suggests that another refugium existed near these localities in southeastern British Columbia. This region is thought to have been completely covered by ice during the Wisconsinian glaciation (Matthews 1979b), so the refugium would likely have been located in Washington or Idaho, just south of the Wisconsinian ice sheet. Present-day populations of Pfs-c in eastern British Columbia would thus be descended from individuals which migrated north as the ice receded.

Presence of a southern refugium is supported by Kavanaugh's (1988) study of the carabid genus Nebria. He found that present-day continental populations were descended primarily from southern, rather than coastal refugia, probably because coast-adapted populations were outcompeted in continental environments. Similarly, island populations of Pfs-c may have been outcompeted by southern migrants in the colonization of newly-exposed mainland habitats.

This evidence that an inland refugium existed for Pfs-c supports the hypothesis that Pfs-c is subdivided into two groups. Such subdivision of Pfs-c would have occurred more recently than the separation of the coastal and inland forms of Pfs. Again, further work on larger populations is necessary to fully understand the substructuring within the Pfs-c taxon, so at present nothing more can be inferred.

3.4.2 P. stygicus Populations

All sampled allopatric populations of Pst were electrophoretically and morphologically very similar. Ecologically, these populations were similar as well. All allopatric Pst sites

for which ecological descriptions were known had Carex and Salix around their margins. Pst lives in the Carex clumps, and around the willow roots, so obviously these plants are crucial components of their habitat. These ponds occur in mixed boreal and parkland forests. This concurs with the results of Carter (1971), who studied the ecological aspects of Pst and other Patrobus species at George Lake. He found that Salix, Carex aquatilis Wahlenb., and C. rostrata Stokes dominated Pst habitat there.

The similarity of electrophoretic, morphological and ecological characters indicates that these populations are closely related evolutionarily, and clearly form a homogeneous entity. Unlike Pfs, all known Pst individuals are macropterous. As a result, the species is more vagile, which may be the reason no substructuring was found in Pst, despite its extensive range.

The range of Pst extends across Canada east of the Pacific coast, and south into Washington and the Rocky Mountain states. This distribution pattern has been seen in many other insects (Munroe 1956), and is typical of organisms adapted to a boreal environment.

3.4.3 Comparison of P. stygicus to P. fossifrons

Pst and the two morphs of Pfs differ in the shape of their elytral microsculpture, and in the shape of the median lobe of males. These characteristics are depicted in the previous chapter (Figures 2.7 and 2.8). Elytral microsculpture is the characteristic used in Darlington's (1938) and Lindroth's (1961) keys to distinguish these species. Most specimens have clearly isodiametric or clearly transverse microsculpture. Pfs-c is slightly larger, and Pfs-i is considerably larger, based on pronotal length, than Pst. Both morphs of Pfs also have relatively wider pronota, based on pronotal width minus length, than Pst. These pronotal differences are illustrated in Figure 3.11. Pfs-i have longer antennal tips, but Pfs-c have tips of about the same size as Pst. Pfs-c have less elongate antennal segments than Pst. Pfs-i antennomeres varied from less elongate, to as elongate as those of Pst. Typical antenna tips of the species and morphs appear in Figure 3.12. Both morphs of Pfs have relatively shorter R_1 wing veins, and relatively wider wing wedge cells, than Pst (Figure 3.13). Because of their geographical variation, the latter two characters do not help much to distinguish Pfs and Pst in allopatry. However, they were useful in analysis of sympatric areas.

None of the differences noted above are absolute; some specimens are within the range of both species for any of these characters. Thus, one must use several characters to identify intermediate specimens. The elytral microsculpture, male median lobes, and PWL

are useful characters to separate most Pfs from Pst. As well, ALN and ASQ, examined in conjunction, independently separate the two Pfs morphs from Pst. In conjunction with fixed electrophoretic differences at *Ao*, *Est-1*, and *Odh*, these characters separated all of the allopatric specimens.

Thorpe (1979, 1983) reported that insect species in the same genus typically have Nei's genetic identities of 0.25 to 0.85. The values for Curlew, the only large allopatric Pfs population sample which was studied electrophoretically, when compared to the allopatric Pst population samples Chain, George, Long, and Hinton (Table 3.2) were all within this range. Thorpe also reported that populations within the same species tended to have genetic identities of 0.9 or greater. The values for Curlew Pfs-i, compared to Pst populations, are well below that, indicating that Pfs and Pst are quite distinct.

The morphological and genetic differences suggest that Pfs and Pst have been isolated for quite some time. They probably diverged some time in the Miocene (five to 20 million years ago), when major environmental changes were occurring in North America. This put great evolutionary pressure on organisms there, and is thought to be responsible for the origin of many extant insect species (Matthews 1980). These changes are probably responsible for the divergence of Pfs and Pst as well.

3.5 Summary

Two morphs of Pfs are described, Pfs-i and Pfs-c. Pfs-c may be divided further, but numbers of specimens were inadequate for full exploration of this possibility. These morphs are hypothesized to have diverged during the Nebraskan glaciation, 1.5 million years ago. More recently, Pfs-i appear to have recolonized northern North America from a glacial refugium south of the ice sheets, rather than from the Cypress Hills. The most recent refugia for Pfs-c are hypothesized to have existed south of the ice sheets, and on Kodiak Island.

All allopatric populations of Pst were very similar morphologically, genetically, and ecologically. The greater homogeneity of Pst may be because all individuals are long-winged, unlike Pfs. Thus the species is more vagile, and populations are less likely to be isolated from one another.

Morphological, electrophoretic, and ecological differences between Pst and the two morphs of Pfs are summarized in Table 3.3. In allopatry, Pst and Pfs are quite distinct. Though no single character can be used to distinguish these taxa, they can be separated by using several characters. The allopatric populations are pooled in these three taxa, for comparison to sympatric populations of the Pfs/Pst complex.

Table 3.1: Qualitative morphological characters of allopatric populations. Numbers are character state ranges; averages of variable populations are in brackets. Elytral microsculpture is coded as '1' (isodiametric) through '5' (transverse); median lobes of males are coded as '1' (spoon shaped) through '5' (fish-hook shaped); wing development is coded as '0' (brachypterous) or '1' (macropterous); and the secondary sclerotized island is coded as absent ('0'), present ('1'), or present but attached, deformed, or tiny ('a', 'd', and 't', respectively). 'na' indicates where characters were not applicable, and 'ns' indicates where characters were not scored.

SPECIES / POPULATION	MICRO-SCULPTURE	MEDIAN LOBE	WING DEVELOPMENT	SECONDARY SCLEROTIZED ISLAND
Patrobus fossifrons:				
Kodiak I.	1	1	0*	na
Vancouver I.	1	1-2 (1.29)	0-1 (0.05)	0-1 (0.13)
Manning	1	na	1	0
Creston	1	1	1	0
Deep	3	ns	0	na
Train	1	ns	0	na
St. Mary	1	1	0	na
Crowsnest	1	1-2 (1.60)	0*	na
Curlew	1	ns	1	a,1 (0.94)
Pothole	1-2 (1.36)	ns	1	1
Del Bonita	2	na	1	a (0.50)
Beaver	1	1-2 (1.50)	0-1 (0.29)	1
Arco	1	1-2 (1.33)	0-1 (0.08)	0,a (0.25)
P. stygicus:				
Circle	3-5 (3.83)	4-5 (4.80)	1*	ns
Williams L.	3-5 (3.84)	5	1*	ns
Long L.	3-5 (4.29)	ns	1	0,a,t (0.02)
George L.	3-5 (4.79)	5	1	0,t,1 (0.03)
Hinton	4-5 (4.80)	ns	1	0,d,t (0.11)
Kananaskis	5	5	ns	ns
Columbia L.	3-4 (3.21)	4-5 (4.88)	ns	ns
Island	3	5	ns	ns
Cranbrook	3-5 (3.90)	4-5 (4.32)	ns	ns
Wardner	3-4 (3.50)	5	ns	ns
Chain	3-5 (4.65)	5	1	0,t (0.01)
Blakiston	5	ns	1	0

* based on Lindroth's (1961) observations of the same specimens.

Table 3.2: Nei's (1978) unbiased genetic identities between allopatric populations.

SPECIES AND POPULATION	1	2	3	4
1. 'inland' Patrobus fossifrons Curlew	----			
2. P. stygicus Long	.399	----		
3. P. stygicus George	.400	1.0	----	
4. P. stygicus Hinton	.405	1.0	1.0	----
5. P. stygicus Chain	.413	.997	.998	.999

Table 3.3: Summary of characteristics of 'coastal' and 'inland' Patrobus fossifrons, and P. stygicus, in allopatry. For electrophoretic loci, bold letters signify fixed alleles, normal letters signify common alleles (frequency ≥ 0.25), and letters in brackets signify less common (frequency < 0.25) alleles.

CHARACTERS	'coastal' P. fossifrons	'inland' P. fossifrons	P. stygicus
Morphological:			
microsculpture	isodiametric	isodiametric	transverse
median lobe	spoon	spoon	fish-hook
wing length	dimorphic	dimorphic	macropterous
sclerotized island	usually absent	usually present	usually absent
pronotum shape	med. length, wide	long, wide	short, narrow
antenna shape	short, narrow	long, wide	short, wide
R_1 wing vein	short	short	long
wing wedge cell	wide	wide	narrow
Electrophoretic:			
Ao	**G**	**F**	**E**
Apk	B, C	**C**	C (D)
Est-1	C, E	E (C)	A (B, F)
Est-3	K, L (I)	K, M	J (G, L)
Odh	F, H	H (F)	G (C, E, J)
Pgi	B, E	**B**	E (B, I)
Pgm	D, I	D (B)	D (B, G, I)
Ecological:			
habitat	boreal and parkland marshes & river margins	prairie marshes	boreal and parkland marshes

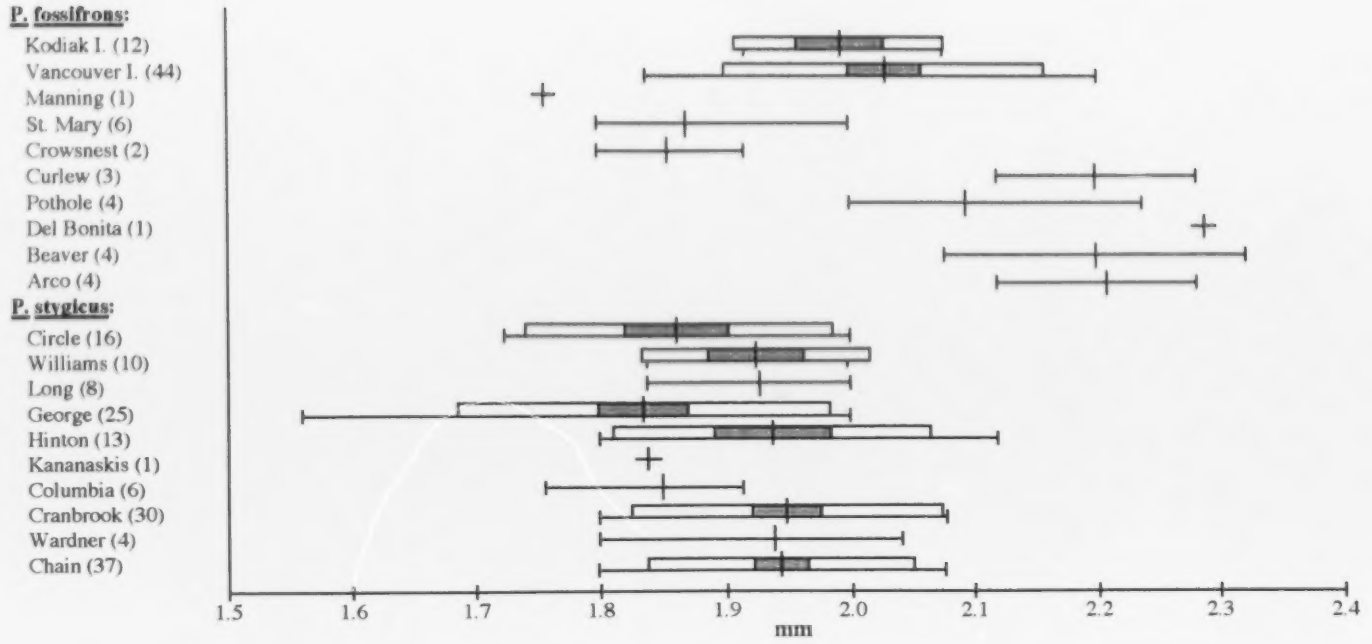


Figure 3.1. Pronotal length of females from allopatric Patrobus populations. Sample sizes are in brackets. Based on the procedure of Hubbs and Hubbs (1953)*.

* Horizontal line = range of measurements; vertical line = mean; outlined box = mean ± 1.5 standard deviations; shaded box = mean ± 2 standard errors.

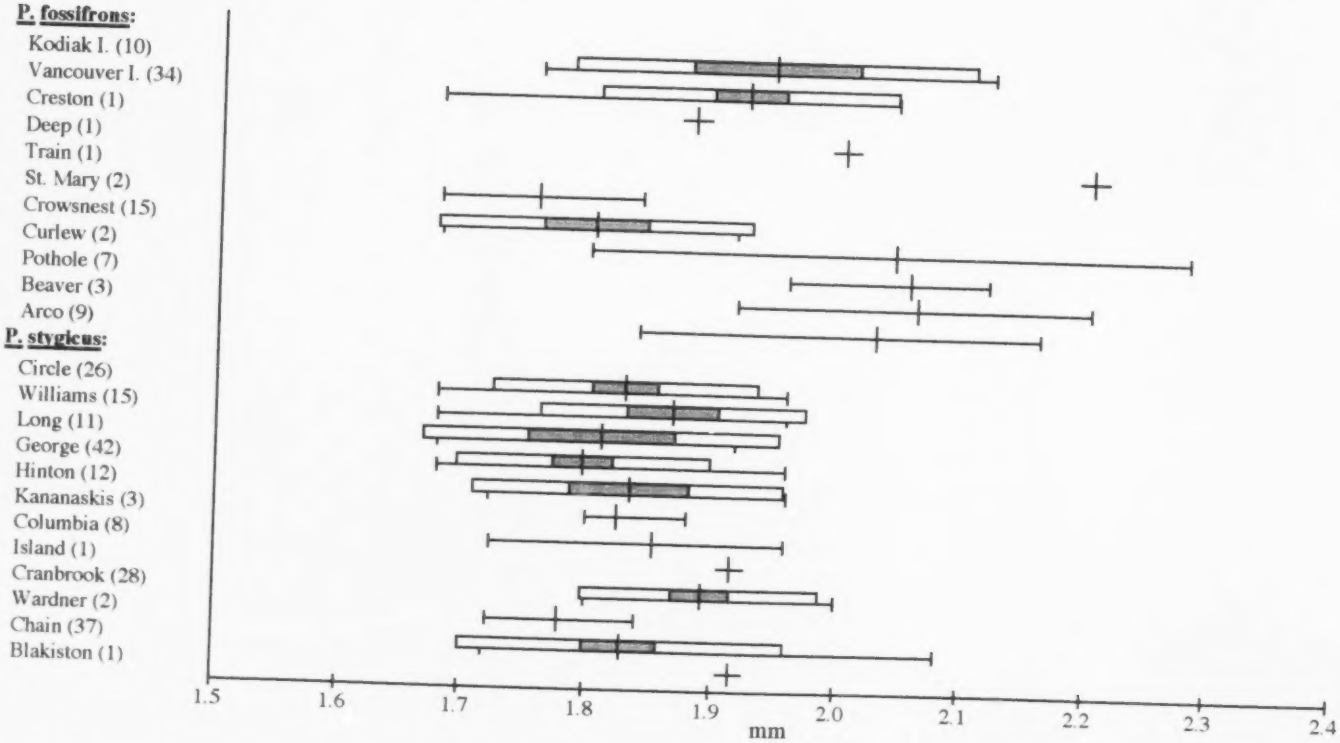


Figure 3.2. Pronotal length of males from allopatric Patrobus populations. Sample sizes are in brackets. Based on the procedure of Hubbs and Hubbs (1953)*.

* Horizontal line = range of measurements; vertical line = mean; outlined box = mean ± 1.5 standard deviations; shaded box = mean ± 2 standard errors.

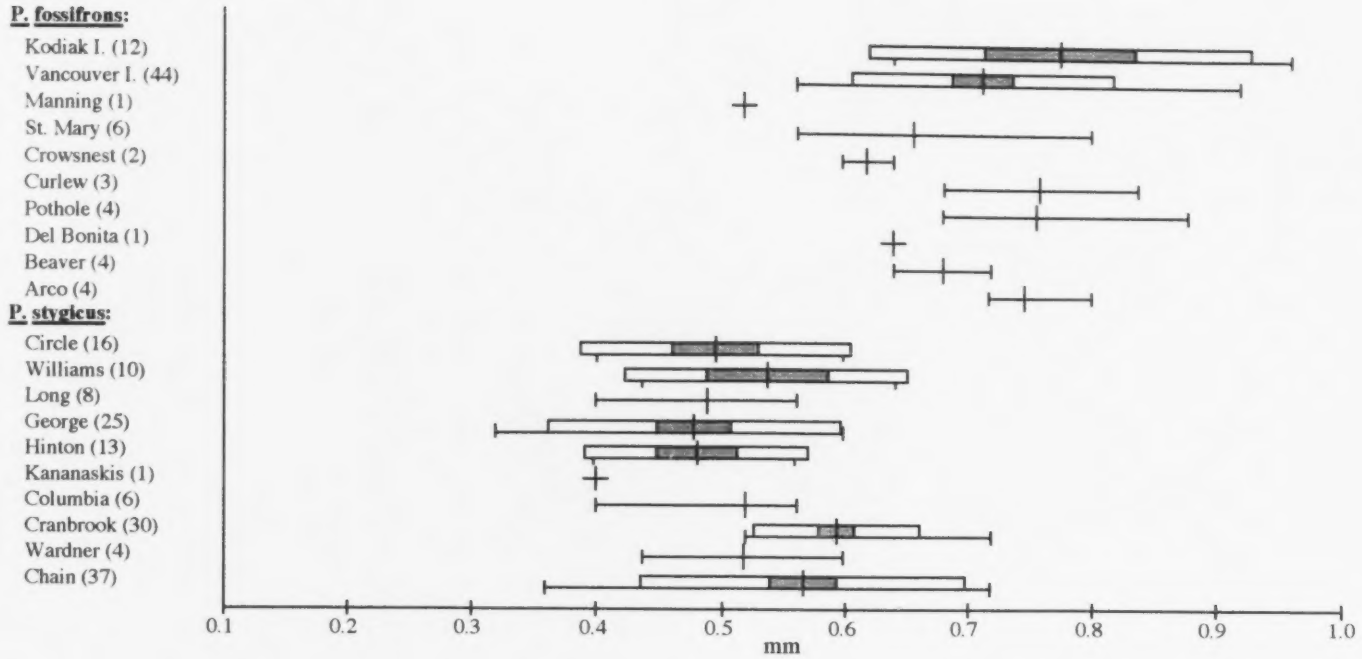


Figure 3.3. Pronotal width minus length of females from allopatric Patrobus populations. Sample sizes are in brackets. Based on the procedure of Hubbs and Hubbs (1953)*.

* Horizontal line = range of measurements; vertical line = mean; outlined box = mean ± 1.5 standard deviations; shaded box = mean ± 2 standard errors.

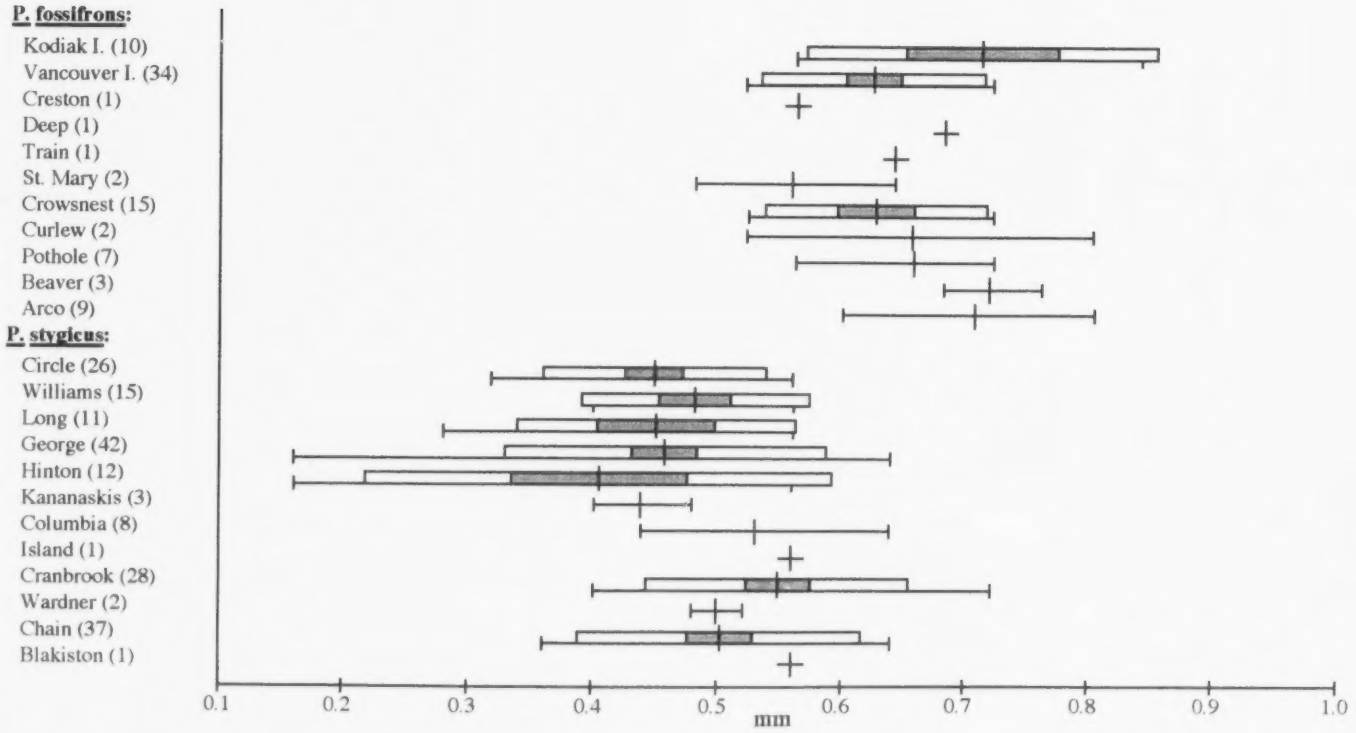


Figure 3.4. Pronotal width minus length of males from allopatric Patrobus populations. Sample sizes are in brackets. Based on the procedure of Hubbs and Hubbs (1953)*.

* Horizontal line = range of measurements; vertical line = mean; outlined box = mean ± 1.5 standard deviations; shaded box = mean ± 2 standard errors.

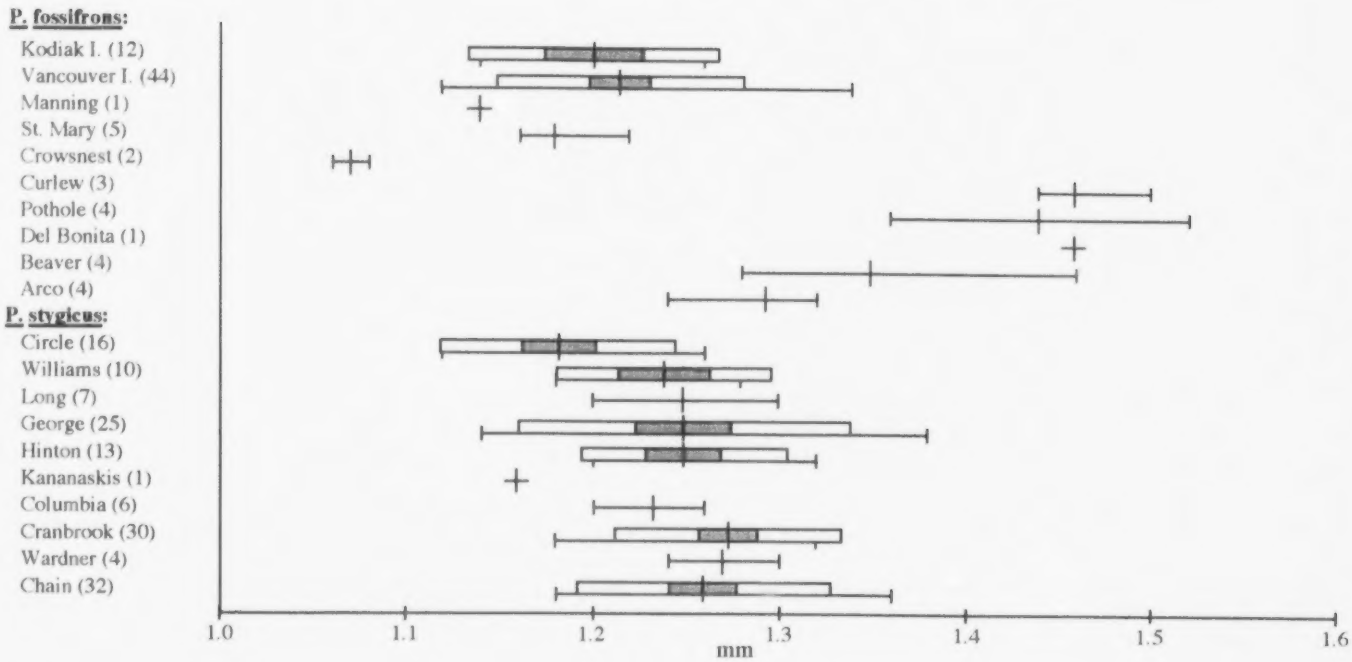


Figure 3.5. Antenna tip lengths (sum of length of antennomeres nine, 10, and 11) of females from allopatric Patrobus populations. Sample sizes are in brackets. Based on the procedure of Hubbs and Hubbs (1953)*.

* Horizontal line = range of measurements; vertical line = mean; outlined box = mean ± 1.5 standard deviations; shaded box = mean ± 2 standard errors.

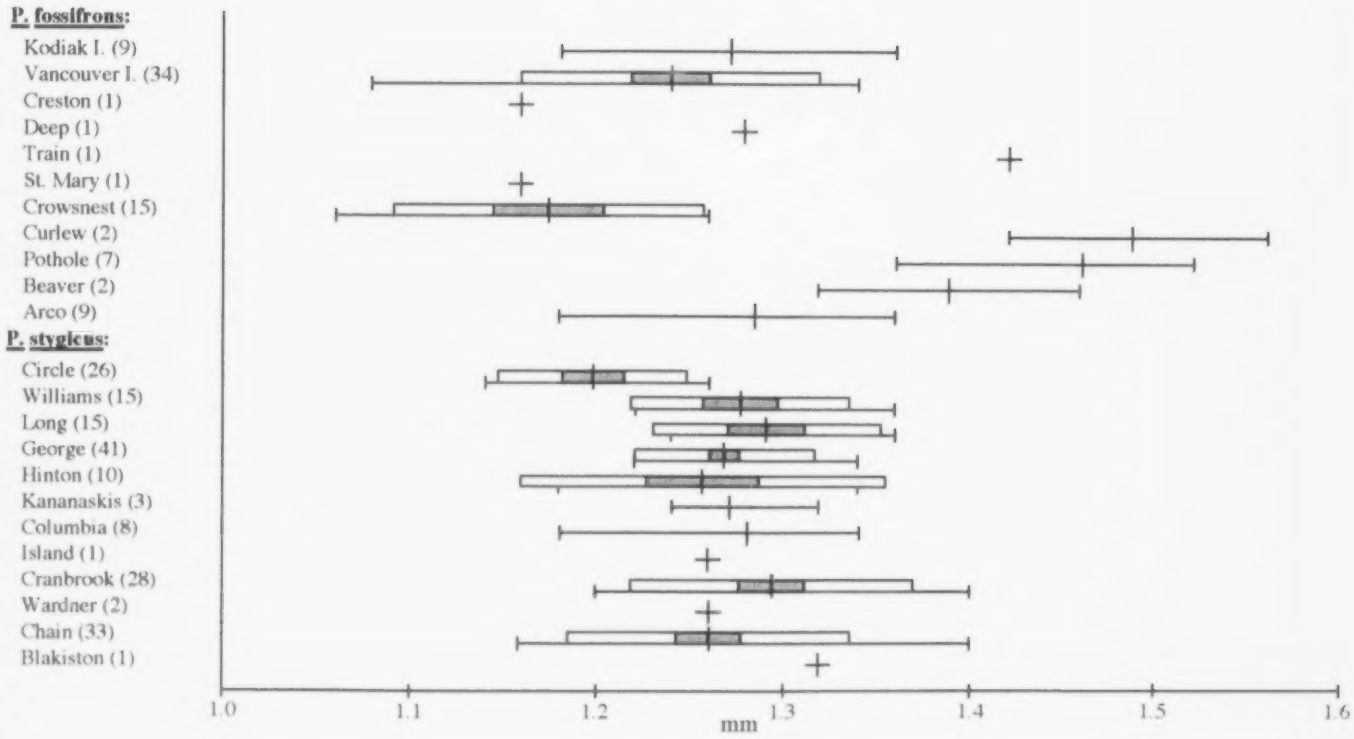


Figure 3.6. Antenna tip lengths (sum of length of antennomeres nine, 10, and 11) of males from allopatric Patrobus populations. Sample sizes are in brackets. Based on the procedure of Hubbs and Hubbs (1953)*.

* Horizontal line = range of measurements; vertical line = mean; outlined box = mean ± 1.5 standard deviations; shaded box = mean ± 2 standard errors.

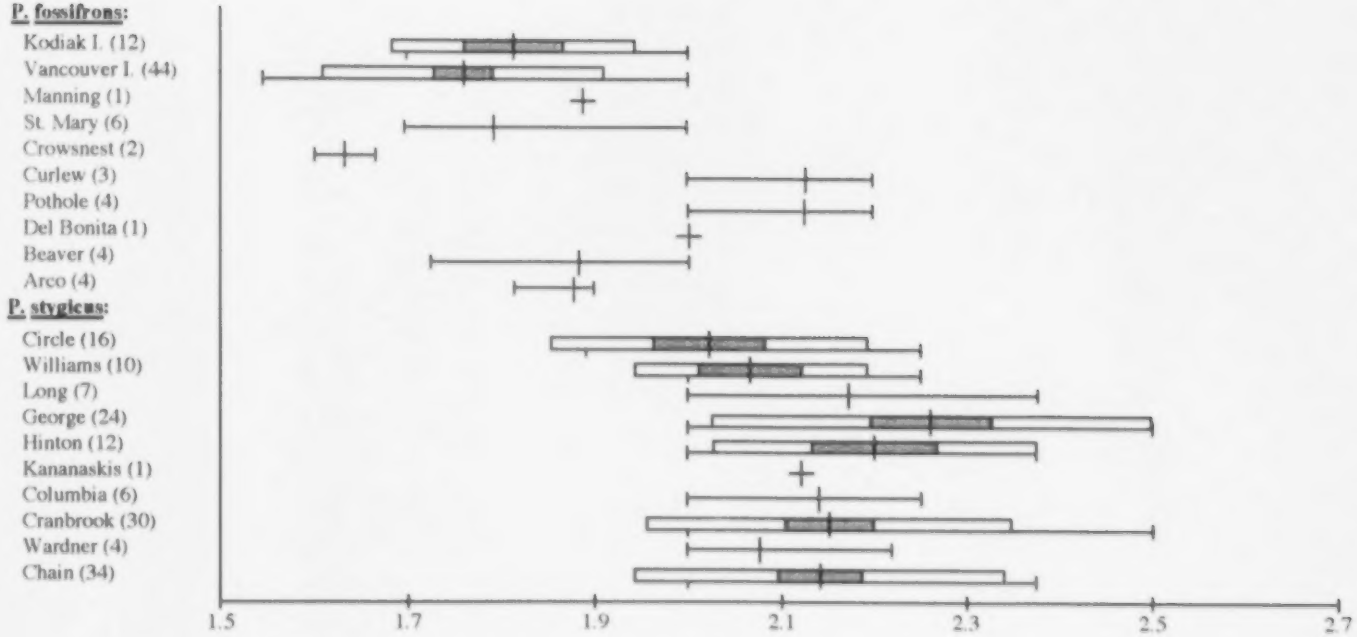


Figure 3.7. Ninth antennomere squareness (length divided by width) of females from allopatric Patrobus populations. Sample sizes are in brackets. Based on the procedure of Hubbs and Hubbs (1953)*.

* Horizontal line = range of measurements; vertical line = mean; outlined box = mean ± 1.5 standard deviations; shaded box = mean ± 2 standard errors.

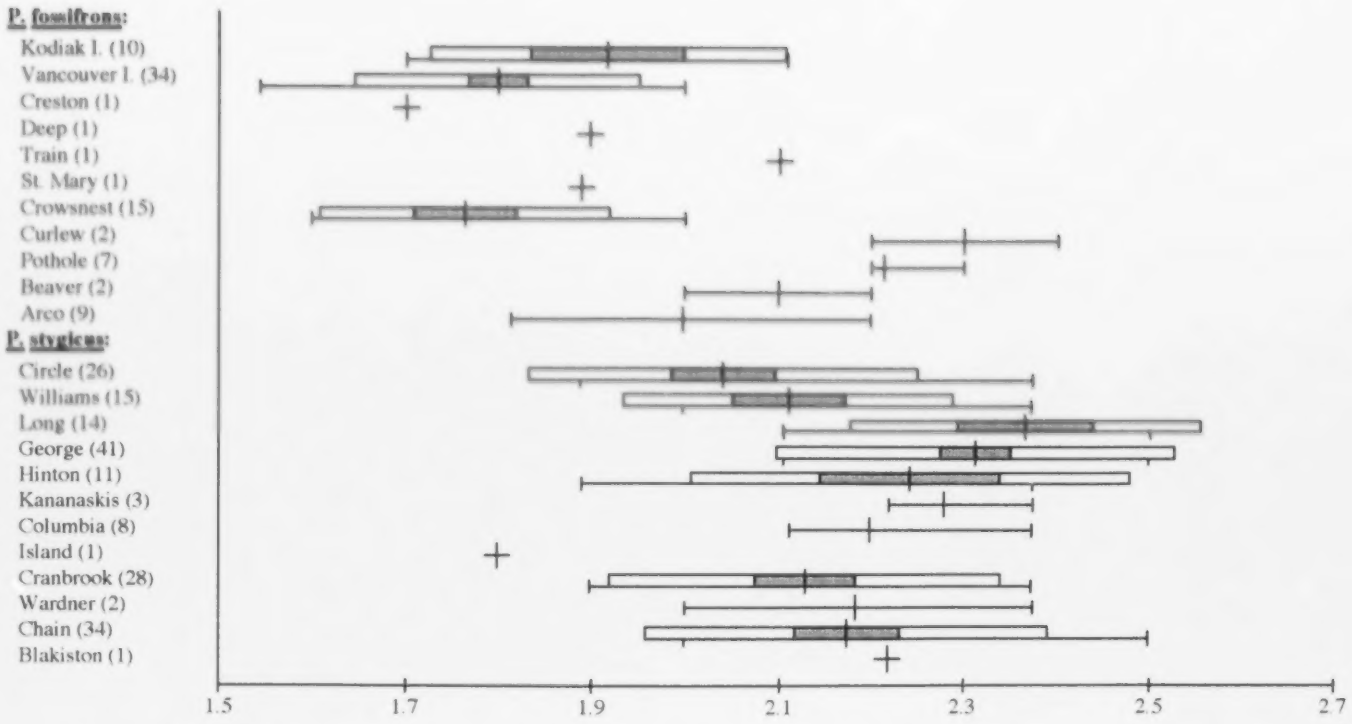


Figure 3.8. Ninth antennomere squareness (length divided by width) of males from allopatric Patrobus populations. Sample sizes are in brackets. Based on the procedure of Hubbs and Hubbs (1953)*.

* Horizontal line = range of measurements, vertical line = mean; outlined box = mean ± 1.5 standard deviations, shaded box = mean ± 2 standard errors.

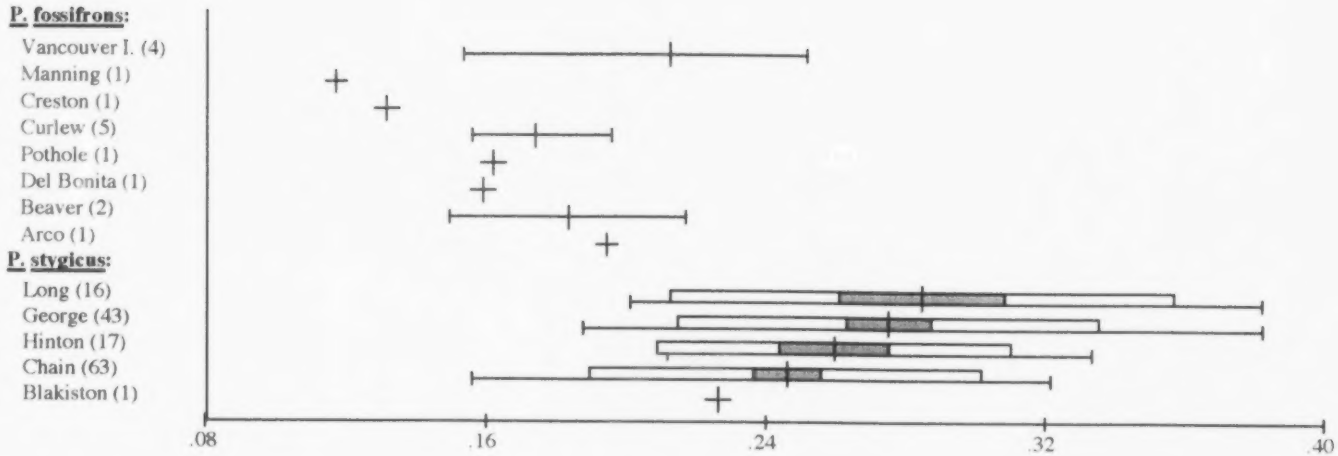


Figure 3.9. Relative length of R_1 wing vein, in allopatric Patrobus populations. Sample sizes are in brackets. Based on the procedure of Hubbs and Hubbs (1953)*.

* Horizontal line = range of measurements; vertical line = mean; outlined box = mean ± 1.5 standard deviations; shaded box = mean ± 2 standard errors.

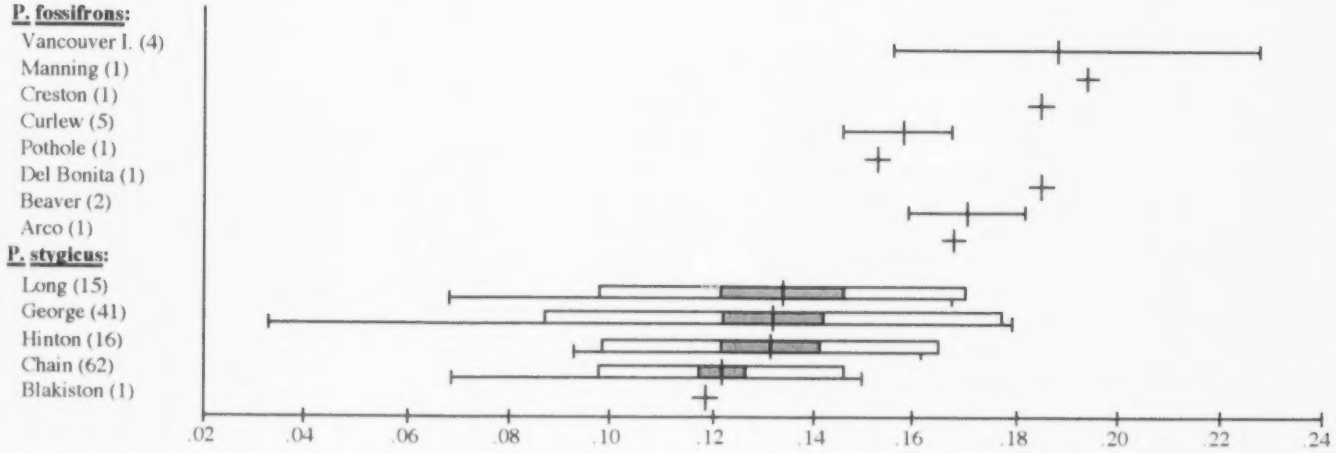


Figure 3.10. Relative width of wing wedge cell, in allopatric Patrobus populations. Sample sizes are in brackets. Based on the procedure of Hubbs and Hubbs (1953)*.

* Horizontal line = range of measurements; vertical line = mean; outlined box = mean ± 1.5 standard deviations; shaded box = mean ± 2 standard errors.

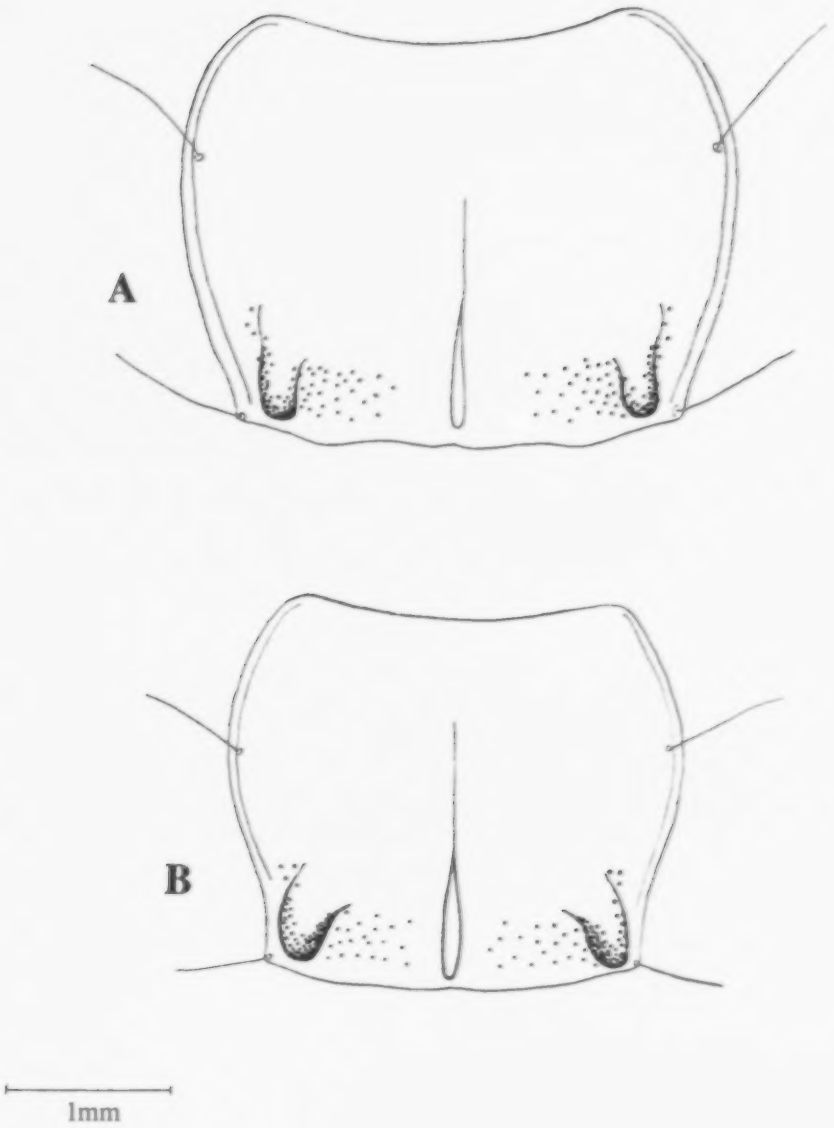


Figure 3.11: Typical pronota, dorsal aspect, of Patrobus fossifrons (A) and P. stygicus (B).

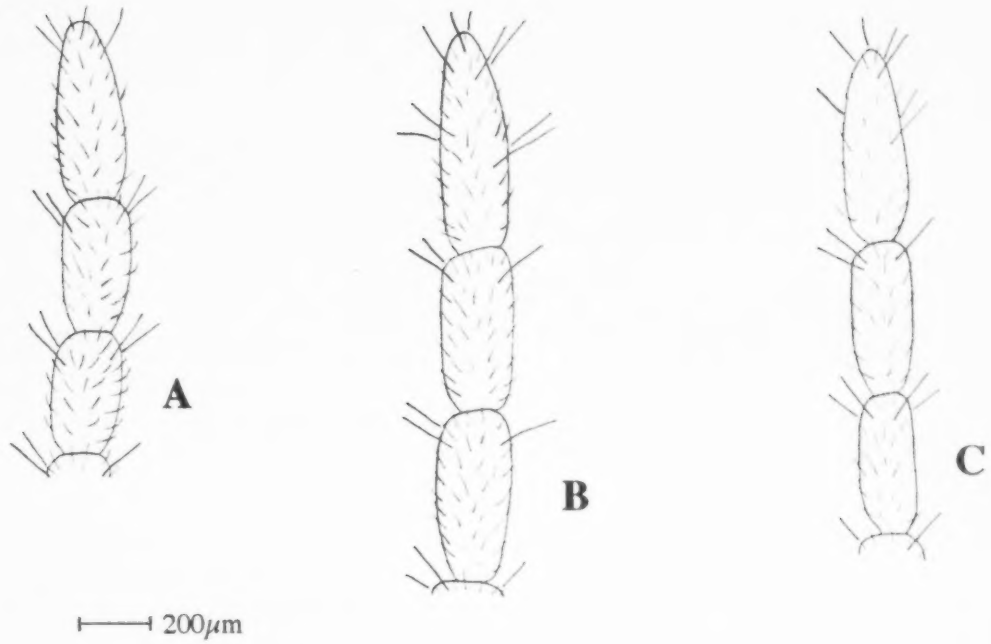


Figure 3.12: Typical antenna tips, anterior aspect, of 'coastal' Patrobus fossifrons (A), 'inland' P. fossifrons (B), and P. stygicus (C).

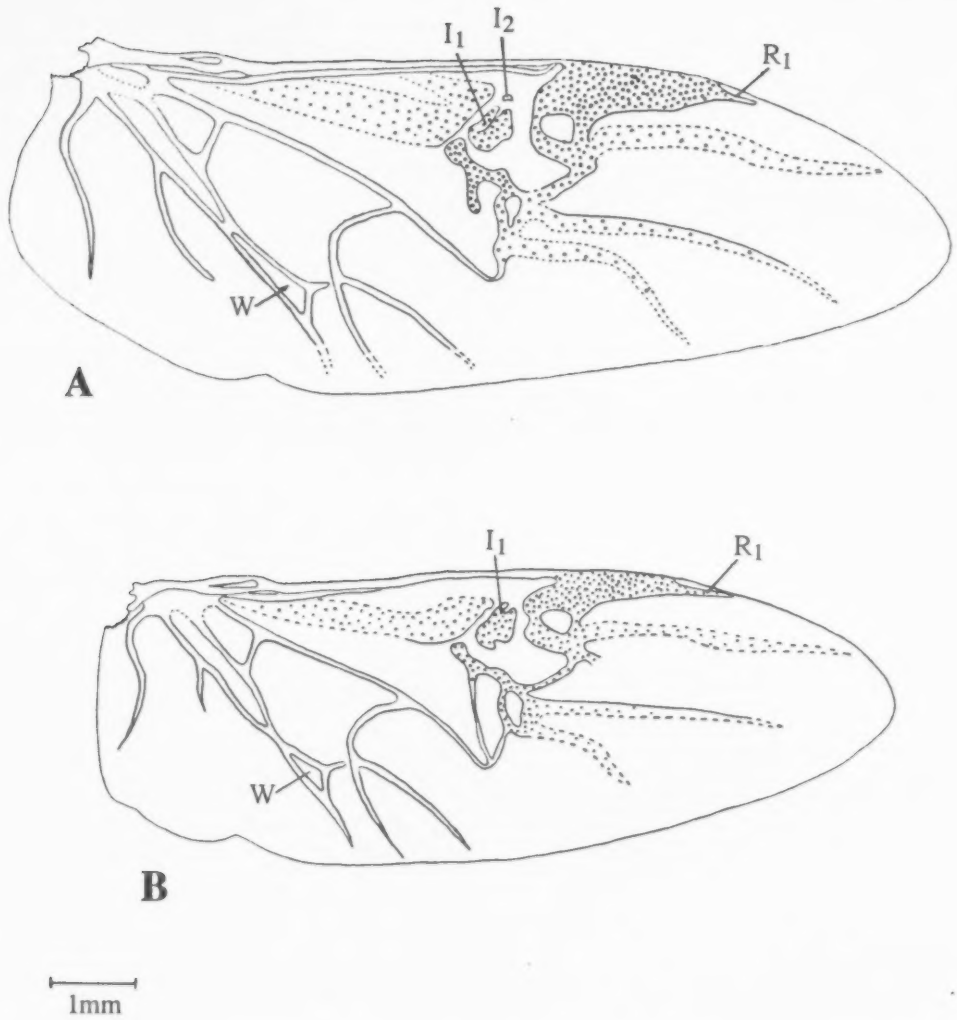


Figure 3.13: Typical flight wings of P. fossifrons (A) and P. stygicus (B). I_1 and I_2 are the primary and secondary sclerotized islands, respectively; R_1 is the first branch of the radial vein; and W is the wedge cell.

4. EXAMINATION OF SYMPATRIC ZONES AND HYBRIDS

4.1 Introduction

P. fossifrons (Pfs) and P. stygicus (Pst) have been found within 25 km of each other in three areas in western North America; the Pincher Creek area of southwestern Alberta (Figure 4.1), the Cypress Hills on the Alberta-Saskatchewan border, and at Wauconda, Washington (Figure 2.1). Characteristics of these sympatric specimens are compared to the character states of allopatric Pfs and Pst, which were described in Chapter 3.

This study is organized as follows. In this chapter I determine which species are present at the various sites, examine ecological differences between these sites, and identify some possible hybrid specimens. In Chapter 5, I compile indirect evidence for hybridization, and calculate estimates of gene flow.

4.2 Sympatric Zone Data Examined

Samples from the allopatric populations discussed in Chapter 3 have been pooled together to provide baseline species characteristics, for comparison to the sympatric specimens.

Specimens in the zone of sympatry were identified as 'inland' Pfs (Pfs-i), 'coastal' Pfs (Pfs-c), or Pst using the morphological and electrophoretic characters discussed in Chapter 3. Intergrades between Pfs and Pst, identified as such and discussed below, are treated in this chapter as probable hybrids, and have been separated in the following figures and tables.

Qualitative morphological characters of sympatric Pfs and Pst appear in Table 4.1. The quantitative characters for these population samples are summarized as Hubbs-Hubbs diagrams (Figures 4.2 to 4.11). Refer to Chapter 2 for a discussion of the interpretation of these plots based on Hubbs and Hubbs (1953).

Electrophoretic data are summarized in Table 4.2. For more detailed information, refer to Tables 2.5 and 2.6, or Appendix #4. Ecological descriptions of the various ponds appear at appropriate points throughout the chapter.

4.3 Results and Interpretations

4.3.1 Pincher Creek, Alberta

The prairie region of southern Alberta provides the habitat of Pfs-i. Between Pincher Creek and the Waterton River, these prairies meet the boreal zone with little or no transition zone. Patrobus was found at several sites in this area (Figure 4.1).

Morphological Results

According to morphological characters (Figures 4.2 to 4.11), Tern Pond specimens and the two pinned specimens from Bull Pond #1 are Pfs-i. The beetles from Antenna, Pecten, Lost Road, and Bull #2 Ponds are Pst.

At Rock Pond, all the specimens except one were clearly Pfs-i. The exception is probably a hybrid, and is separated from the others on the tables and figures. It had Pst microsculpture, Pfs-i character states for PWL and ALN, and intermediate states for the median lobe and for PLN. It was brachypterous, which is highly unusual for either species in this area. This specimen represents 1.4% of the Rock Pond sample.

All but one of the Ball specimens are Pfs-i. That specimen, originally identified as a Pst, has Pst microsculpture and median lobe shape, and Pfs-i PWL and ALN character states. For PLN, its character state is within the range of both species. It is also considered to be a probable hybrid, so it is kept separate in the tables and figures. Like the unusual Rock Pond specimen, it is brachypterous. The Ball hybrid specimen represents 2.7% of the Ball sample. Together, the two hybrid specimens make up 0.67% of the entire Pincher Creek sample.

Lynch Lakes and Kavanaugh's Pond contained both Pfs-i and Pst[1].

The flight-wing characters R1 and W are more consistent at Pincher Creek, since geographical variation is not a factor over such short distances. There is a major difference in R1, and a slight, though significant difference in W, between the two species here. Two other characters that were variable for allopatric beetles, flight wing development and secondary sclerotized island presence/absence, also were more consistent in the Pincher Creek area. Here, all Pfs-i were macropterous, and a very high proportion had the secondary sclerotized island. Pst, (all specimens macropterous), had a very low incidence of the secondary sclerotized island.

[1] Originally, two specimens from Kavanaugh's Pond were thought to be intermediate, so they were given hybrid identification codes. However, examination of all the morphological characters shows that one is a Pst and the other is a Pfs-i. These specimens were only outliers for the microsculpture character.

Electrophoretic Results

Fixed differences at the *Ao* locus (Table 4.2) show that the Tern Pond specimens are Pfs-i. All electrophoresed specimens from Rock Pond also were electrophoretically pure Pfs-i. However, the questionable Rock Pond specimen died and partly decomposed en route to the lab, so it could not be electrophoresed. Antenna, Lost Rd., and Pecten Pond beetles were electrophoretically pure Pst. Electrophoretic data from the Lynch Lakes locality showed that it contained both species. Various fixed alleles in these populations indicate that they are pure stock.

Four new rare alleles were found in the Pincher Creek Pfs-i populations. Their presence is not surprising, since the allopatric samples of Pfs-i were small, and thus these alleles could easily have been present there and simply missed.

Ecological Results

Tern Pond is on the prairies, several kilometers from the nearest forested area. Antenna, Pecten, and Lost Road Ponds are in semi-open forests, and Lynch Lakes, the Bull Ponds, and Rock Pond are right on the margin between prairie and foothills (Figure 4.1). In fact, Bull Pond #1, a Pfs-i site, is on the prairie, while 500m away, Bull Pond #2, a Pst site, is in the first significant patch of trees as the prairie is replaced by the forest.

The Lynch Lakes site is made up of two ponds about 50m apart. The more northeasterly pond has no trees around it, but the more southwestern one is surrounded on three sides by willows. As at Bull Pond #2, these are the first significant trees of the foothills. The northeast pond contained a large, rather coarse species of Carex, with no moss or Scirpus present. The southwest pond had Scirpus in the center. Its three treed sides contained a short, fine species of Carex, with moss present in the Carex clumps. The open east side of this pond contained larger Carex, similar to that found at the east pond.

The first day at Lynch Lakes, I did not realize that I was collecting both species. However, when I returned, I kept beetles from the different habitats separate, and found only Pst in the moss and short Carex of the treed area of the southwest pond, both Pst and Pfs-i in the coarser Carex at the eastern end of this pond, and only Pfs-i at the open northeast pond. So, even though this is a sympatric site, there appears to be habitat partitioning between Pst and Pfs-i. Though they undoubtedly encounter one another, they tend to live in different microhabitats.

Kavanaugh's Pond could not be shown precisely in Figure 4.1. This site was sampled by D. R. Kavanaugh in 1970, and according to his label data, it is probably Lynch Lakes.

If it is not, then it is a pond very close to Lynch Lakes. In any event, Kavanaugh's Pond is also in the transition zone between prairie and forest.

Ball's Pond also could not be located precisely, but it is in the transition zone as well. According to G. E. Ball, who collected the specimens in 1956, this pond is not Rock Pond or one of the Bull Ponds, but it is somewhere along highway #6, immediately south of these sites. It is probably one of the ponds indicated in Figure 4.1. In 1989, these ponds were highly disturbed by cattle, so they did not support Patrobus populations. No other ponds in the immediate vicinity contained suitable habitat at that time, so it is concluded that Ball's Pond, wherever it is, is no longer a viable Patrobus site.

Based on the ecological descriptions of ponds in the Pincher Creek area, it is clear that Pfs-i ponds are in the prairie, and Pst ponds are in the forested foothills.

Summary

According to morphological and electrophoretic data, some populations in the Pincher Creek sympatric area are Pfs-i, some are Pst, and a few contain both species. Two specimens that were not electrophoresed were morphological intergrades between the two species. Ecological data indicate that Pfs-i and Pst sites are ecologically different. Generally, Pst sites are at higher elevations in forested foothills, while Pfs-i sites tend to be at lower elevations, on treeless prairie. The truly sympatric sites occur right on the margin between these habitats. At Lynch Lakes, a sympatric site, there was evidence of habitat partitioning, as the two species were found in slightly different microhabitats.

4.3.2 Cypress Hills, Alberta

Material from the Cypress Hills consists of a series of pinned beetles collected in the '50s and '60s by G. E. Ball. The majority of specimens are from Elkwater Lake, Alberta, which has extensive Carex and Typha fields at its eastern end. In 1989, extensive searching there did not yield any more Patrobus, though suitable-looking habitat was found. Several suitable-looking marshes existed on the Saskatchewan side of the Cypress Hills in 1989, but no Patrobus were found at that time.

No electrophoretic information was obtainable from these museum specimens. However, morphological characters (Table 4.1 and Figures 4.2 to 4.11) indicate that all of the Cypress Hills specimens are Pst, except for two Pfs-i from the eastern end of Elkwater Lake. These Pfs-i specimens were collected together with four Pst specimens, indicating that the two species co-occurred, both spatially and temporally, in the Cypress Hills.

4.3.3 Wauconda, Washington

Morphological Data

A series of Patrobus was collected from a pond at Wauconda, in north-central Washington, in 1989. This sample contained both Pfs-c and Pst, as well as a single specimen of mixed stock (3.3% of the sample). Structurally, the hybrid specimen appears to be a Pfs-c. Its microsculpture, pronotal length, and pronotal squareness states are all exclusively Pfs-c. Its antennal length and squareness states could fit into either Pfs-c or Pst. It is brachypterous, like all Wauconda Pfs-c[2]. However, electrophoretic data show that it is clearly of mixed ancestry (see below).

The Hubbs-Hubbs plots (Figures 4.2-4.11) indicate that purebred Wauconda Pfs-c are somewhat larger than allopatric Pfs-c, as indicated by PLN. Differences between Pfs-c and Pst in the character ALN were in the opposite direction from those in allopatry. I have no explanation for this. For the rest of the morphological characters, Pfs-c and Pst at Wauconda generally showed the same characteristics as allopatric Pfs-c and Pst.

Electrophoretic Data

Electrophoretic data (Table 4.2) indicate that, except for the putative hybrid specimen, the Wauconda beetles are pure Pfs or Pst. The Pfs are clearly of the coastal morph, based on the fixation of allele G in the *Ao* locus. Wauconda Pfs alleles at *Odh*, *Pgi*, and *Pgm* also fit the pattern for Pfs-c much better than that of Pfs-i. A comparison of Wauconda Pfs to individual allopatric Pfs-c (Table 2.5) shows that there are allelic differences between Wauconda Pfs and all three allopatric Pfs-c specimens. This supports the idea, discussed in Chapter 3, that there is some subdivision within Pfs-c which cannot be determined with the data at hand.

The putative hybrid specimen is not an F_1. This beetle had one locus (*Est-1*) fixed for Pst alleles, one locus (*Odh*) fixed for Pfs-c alleles, and two loci (*Ao* and *Est-3*[3]) which were heterozygous, with a Pst and a Pfs-c allele. The hybrid specimen's alleles at the other four loci were of indeterminate origin, since they were alleles shared by Pfs-c and Pst. Obviously, this specimen is of mixed stock, though it is not an F_1 hybrid.

---

[2] Because brachyptery appears to be a feature of hybrid stock (see section 4.4.3), the fact that this Wauconda specimen is brachypterous in a region where Pfs-c is brachypterous does not help identify it as a Pfs-c.

[3] Electromorph L of the *Est-3* locus is assumed here to be present only in Pst at Wauconda. However, L was seen in allopatric Pfs-c, so there is a slight possibility that L is present at a very low frequency in Wauconda Pfs-c. If that is so, the hybrid specimen could be homozygous for Pfs-c electromorphs at *Est-3*.

Ecological Data

The Wauconda area is primarily dry pine forest in rolling hills. The pond itself was surrounded by willow trees, and had extensive Carex patches. All the specimens of both species were collected within a 10m X 10m area, and no subdivision of the pond into microhabitats was evident. Thus, the habitat partitioning evident at Pincher Creek was not apparent at Wauconda.

Summary

Both Pfs-c and Pst inhabit the Wauconda Pond. Electrophoretic analysis proved that one specimen was of mixed parentage, and that all other Wauconda beetles were purebred, though possibly slightly divergent, stocks of their respective species.

4.4 Discussion

4.4.1 Habitat Preferences

An important question raised by examination of the ecological data is, exactly which ecological factors determine the habitats of Pfs-i and Pst? Clearly these species prefer different habitats.

One possibility is that perhaps Pfs-i require more day-degrees than Pst, and/or perhaps Pst cannot survive higher temperatures. In southwestern Alberta, the boundary between Pfs-i sites and Pst sites more-or-less corresponds to the treeline separating the prairies from the foothills. In the Pincher Creek area, this is at approximately 4500 feet (1360m) (see Figure 4.1). The prairie ponds there frequented by Pfs-i receive much more sunlight, and are generally at a lower elevation, so they are undoubtedly warmer. Depending on the elevation, the frost-free period around Pincher Creek varies widely, from 60 to 120 days. Local ranchers have told me that a change in altitude of less than 100m causes a major difference in the climate. Clearly, though the foothills and prairies are close geographically, they are climatically quite different.

Another likely factor is the amount and type of available ground cover for concealment and overwintering. Cover is very important to Pfs and Pst, as shown by the rocks for which Rock Pond is named. At that site, Pfs-i were highly concentrated under the rocks, but they were very scarce around the rest of the pond. Pfs-i ponds either had rocks or no visible cover, whereas Pst ponds always had old logs and debris around them, due to the nearby trees. Perhaps Pfs-i and Pst larvae require different types of cover to survive.

Ecologically, the Cypress Hills area of sympatry is quite similar to the Pincher Creek area. The Cypress Hills are at a similar latitude, and reach an altitude of 1392 m (just over 4500 ft.). They form an island of lodgepole pine forest in the great plains, so they are an island of Pst habitat in a large area of Pfs-i habitat. Elkwater Lake, the Cypress Hills sympatric site, is at an elevation of 1209m (3990 feet), and is right on the margin between forest and prairie. This is the same situation as in the Pincher Creek area, where Pfs and Pst encounter one another at the margin between their respective habitat types.

I do not know what is happening ecologically at Wauconda, Washington, because I have not been able to describe the habitat preferences of Pfs-c and Pst in the region when they are in allopatry. However, this is the farthest south that Pst has been found on the west side of the Rockies, so the area is probably marginal for its survival.

4.4.2 Hybrid Ancestries

A second question is, what are the ancestries of the hybrid specimens? The degree of fertility between taxa, and the fertility of hybrid offspring, can provide an indication of the extent of genetic similarity between the taxa. Thus, details of the types of hybridization events which are possible can tell us about the genetic relatedness of Pfs and Pst.

As discussed above, two probable Pfs-i/Pst hybrids were found in the Pincher Creek area, one from Rock Pond and one from Ball's Pond. A Pfs-c/Pst hybrid was found at Wauconda Pond.

The probable hybrids from Pincher Creek cannot be studied electrophoretically, so their ancestry cannot be traced in that manner. All that can be deduced about hybridization in this area is that successful crosses are quite rare, or else more hybrids would have been found.

There is no reason not to expect hybridization to occur occasionally at Cypress Hills. Based on the rarity of hybrids in the Pincher Creek populations, the Cypress Hills sample may not have been large enough for any hybrids to have been found.

The hybrid from Wauconda was electrophoresed, so much more is known of its ancestry. Obviously, this specimen is of mixed stock, though it is not an F_1 hybrid. The loci for which it is heterozygotic for Pfs-c and Pst alleles (*Ao* and *Est-3*) can be explained by a simple cross of a Pfs-c and a Pst. However, the fact that it is fixed for a Pfs-c allele at *Odh*, and for a Pst allele at *Est-1* cannot be explained by a simple Pfs-c/Pst cross. Those loci indicate that both parents had a Pfs-c allele at *Odh*, and a Pst allele at *Est-1*. Therefore, both parents of this beetle had to have been mixed-stock. Taking this into consideration, the simplest way for this beetle to have been produced is via a sibling mating of two F_1 hybrids (theory A; Figure 4.12).

Haldane (1922) found that, in hybrids, the heterogametic sex is more likely to be sterile or unfit than the homogametic sex, because it has only single copies of alleles for many genes on the sex chromosomes. If this is the case in Patrobus, and male hybrids are sterile, then the ancestry of the Wauconda hybrid becomes somewhat more complex. Again assuming that it is the product of a single hybridization event, the only way to produce the necessary mixed-stock fertile males would be a backcross of a Pfs-c/Pst hybrid female with a male of either species. A male of the resulting F_2 generation then could have produced the hybrid beetle by mating with a female sibling (theory B; Figure 4.12). Therefore, if F_1 males are sterile, the hybrid would have to be at least an F_3.

After examining this entire Pfs-c/Pst sample for electrophoretic characters, and finding only one individual of mixed stock, it is clear that successful hybridization is an unusual event. However, the above hypotheses of the genetics of this hybrid require the invocation of several more unusual events. First, few or no other F_1 hybrids besides the ancestors of this beetle could have successfully reproduced, or else some other trace of mixed ancestry would have been found at Wauconda. Therefore we can assume that very few of these F_1 hybrids existed. Second, to produce the hybrid specimen, these mixed-stock ancestors would have had to have mated with each other rather than with any of the many purebred Pfs and Pst present at the site. Third, few of the 1989 generation (the year that the hybrid specimen was collected) could have survived to maturity, or more would have been found. This is quite a sequence of improbable events. However, if the hybrid beetle is the result of a sibling mating, then obviously those events must have occurred.

Alternatively, the parents of the mixed stock specimen could be the product of two different hybridization events. Then a sibling mating would not have had to have occurred in its ancestry. However, this increases the odds of other mixed stock beetles being produced; none of which were found. The fact that only one specimen out of 30 (3.3%) was of mixed stock suggests that successful hybridization events are somewhat rare. So, the single hybridization theory remains the most likely.

Another, highly speculative explanation is that perhaps this beetle is the product of a hybridization event in which some genetic information was lost in the F_1 generation. In F_1 hybrids, differences between maternal and paternal DNA often cause translocations, inversions, and other copying errors. Perhaps errors of this sort caused one or more alleles to be inactivated or deleted. This means that loci which are in fact heterozygous, or contain a null allele, would be coded as homozygous for the single active allele. This situation would be detectable if the enzyme were a dimer (or a trimer or tetramer) because a pair of equally-stained bands (or a series of three or four bands) would be present in place of the

single band produced by a homozygote. However, if the enzyme were a monomer, this condition would not be detectable.

I postulate that perhaps Pfs/Pst F_2 beetles are missing some genetic information of one of the parents. If this is happening at the *Est-1* locus (which codes for a monomeric enzyme) in the hybrid specimen[4], then it would no longer have to be the product of parents which were both mixed stock. It could be explained as the offspring of an F_1 hybrid and a pure Pfs-c (theory C; Figure 4.12), a much more likely occurrence.

Whatever the exact ancestry of the Wauconda hybrid beetle, it is clear that it is of mixed Pfs-c/Pst stock. It is also clear that, since it is not a direct F_1 hybrid, at least some hybrids, its parent(s), are fertile. It is not known whether or not hybrids can mate with both parental stocks. Perhaps they can mate successfully only with other hybrids. This would help explain at least one of the aforementioned unlikely events. There are many biochemical, structural, and behavioral characteristics which could possibly affect the occurrence and outcome of these matings. Examination of these would make a very interesting follow-up study.

It is interesting that no F_1 hybrids were found. In his study of Carabus hybridization, Mossakowski (1990) also did not find any F_1 hybrids, even though he found that individuals of hybrid stock comprised as much as 16% of the populations studied. This suggests that, in carabids, F_1 hybrids do not live as long as their offspring or purebred beetles.

Unfortunately, the three hybrid specimens found do not provide enough evidence to compare levels of Pst hybridization with Pfs-c to levels between Pst and Pfs-i. All that can be inferred at this point is that successful matings are rare in both cases. The next Chapter, an examination of gene flow evidence and estimates, will shed some light on this subject.

4.4.3 Brachyptery of Hybrid Specimens

Both hybrids from the Pincher Creek area were brachypterous, in an area where both parental stocks were macropterous. Since brachyptery is phenotypically dominant in carabids (Lindroth 1946, Den Boer *et al.* 1980), the brachypterous allele does not appear to be present in this area. Therefore, the brachyptery of hybrids is likely due to a developmental error caused by incompatible DNA. The hybrid specimen from Wauconda is

[4] If this had been so for the *Odh* locus, which is homozygotic for Pfs-c alleles, it would have been detected, because *Odh* codes for a dimeric enzyme. Thus, under this explanation, the hybrid could not have been the product of a hybrid and a pure Pst. Also for this reason, the hybrid beetle could not be an F_1 in electrophoretic disguise.

also brachypterous. This may be due to a dominant allele from the brachypterous Pfs-c parental stock, or due to a developmental error resulting from hybridization.

Mossakowski *et al.* (1986, 1990) found wing abnormalities in Carabus lineatus / C. splendens hybrids in Spain. In those studies, hybrids usually had a wing form intermediate between the forms of the parental species, but some hybrids (proportions not specified) had aberrant wings or strong differences between the left and right wings.

4.5 Summary

Pincher Creek and Cypress Hills support sympatric populations of Pfs-i and Pst. Within the Pincher Creek area, most sites contained one or the other species, but a few contained both species. Two morphological intergrade specimens, probable Pfs-i/Pst hybrids, were found. Ecological differences were found between Pfs-i and Pst habitats. Even at mixed sites, the two species showed signs of habitat partitioning. Differences in climate and ground cover are suggested as possible factors determining where Pfs-i and Pst can live.

The Cypress Hills beetles contained pure stocks of Pfs-i and Pst, with no sign of hybrids. Ecological conditions are similar to those at Pincher Creek.

Wauconda, Washington supports a sympatric population of Pfs-c and Pst. Both species lived there with no evidence of habitat partitioning. One beetle of mixed ancestry was found at this site. This specimen was not an F_1, so at least some hybrids at the site are fertile under at least some conditions.

Table 4.1: Comparison of qualitative morphological characters in sympatric and pooled allopatric populations. Pfs-c = 'coastal' Patrobus fossifrons; Pfs-i = 'inland' P. fossifrons; and Pst = P. stygicus. Numbers are character state ranges; averages of variable populations are in brackets. Elytral microsculpture is coded as '1' (isodiametric) to '5' (transverse); median lobes of males are coded as '1' (spoon shaped) through '5' (fish-hook shaped); wing development is coded as '0' (brachypterous) or '1' (macropterous); and the secondary sclerotized island is coded as absent ('0'), present ('1'), or present but attached, deformed, or tiny ('a', 'd', and 't', respectively). 'na' indicates where characters were not applicable, and 'ns' indicates where characters were not scored.

SPECIES & POPULATION	MICRO-SCULPTURE		MEDIAN LOBE		FLIGHT WING	SECONDARY SCLEROTIZED ISLAND	
POOLED ALLOPATRIC:							
Pfs-c	1-3		1-2		0,1	0,1	
Pfs-i	1-2		1-2		0,1	0,a,1	
Pst	3-5		4-5		1	0,a,d,t,1	
PINCHER CREEK:							
Bull #1 (Pfs-i)	2		1-2	(1.50)	1	1	
Tern (Pfs-i)	1		ns		1	0,t,1	(0.96)
Ball (Pfs-i)	1-3	(1.17)	2-3	(2.32)	ns	ns	
(hybrid)	5		4		0	na	
Kavanaugh (Pfs-i)	1-2	(1.50)	1-2	(1.18)	1	1	
(Pst)	4-5	(4.67)	4-5	(4.75)	1	0	
Lynch (Pfs-i)	1-2	(1.03)	ns		1	a,t,1	(0.97)
(Pst)	4-5	(4.67)	ns		1	0	
Rock (Pfs-i)	1-3	(1.14)	ns		1	1	
(hybrid)	5		3		0	na	
Antenna (Pst)	5		ns		1	0	
Bull #2 (Pst)	5		na		1	0	
Lost Rd. (Pst)	3-5	(4.80)	ns		1	0,a,1	(0.04)
Pecten (Pst)	4-5	(4.88)	ns		1	0	
CYPRESS HILLS:							
Pfs-i	1		2		1	1	
Pst	3-4	(3.48)	3-5	(4.71)	ns	ns	
WAUCONDA:							
Pfs-c	1-3	(1.30)	2		0	na	
hybrid	2		na		0	na	
Pst	4-5	(4.67)	ns		1	0	

Table 4.2: Electrophoretic comparison of sympatric and pooled allopatric populations. Pfs-c = 'coastal' Patrobus fossifrons; Pfs-i = 'inland' P. fossifrons; and Pst = P. stygicus. Bold letters signify fixed alleles, large letters signify common (frequency ≥ 0.25) alleles, and smaller letters signify rare (frequency < 0.25) alleles. Letters for the solitary Antenna and Wauconda hybrid specimens are the actual allele codings.

LOCUS	POPULATION												
	allopatric Pfs-i	Tern (Pfs-i)	Rock (Pfs-i)	Lynch (Pfs-i)	Lynch (Pst)	Antenna (Pst)	Pecten (Pst)	Lost Rd. (Pst)	allopatric Pst	Wauconda (Pst)	Wauconda (hybrid)	Wauconda (Pfs-c)	allopatric Pfs-c
Ao	**F**	**F**	F,G	**F**	**E**	E/E	**E**	**E**	**E**	**E**	E/G	**G**	**G**
Apk	**C**	**C**	**C**	**C**	**C**	C/C	**C**	C,D	C,D	**C**	C/C	**C**	B,C
Est-1	E,C	E,C	E,C	E,C	A,BF	A/A	A,B	A,BF	A,BF	A,F	F/F	E,C	C,E
Est-3	K,M	K,M	K,M	K,M	J,L	J/L	J,L	J,GL	J,GL	J,L	K/L	K,M	K,L,I
Hk	**F**	**F**	**F**	F,G	**F**	F/F	**F**	F,G	F,G	**F**	F/F	**F**	**F**
Odh	H,F	H	H,F	H,F	G,E	G/G	**G**	G,E	G,CEJ	**G**	F/F	F,H	F,H
Pgi	**B**	**B**	**B**	**B**	E,I	E/E	E,B	E,BI	E,BI	E,I	E/E	E,I	B,E
Pgm	D,B	D,A	D,BI	D,B	**D**	D/D	D,G	D,BGI	D,BGI	D,I	I/I	D,I,K	D,I

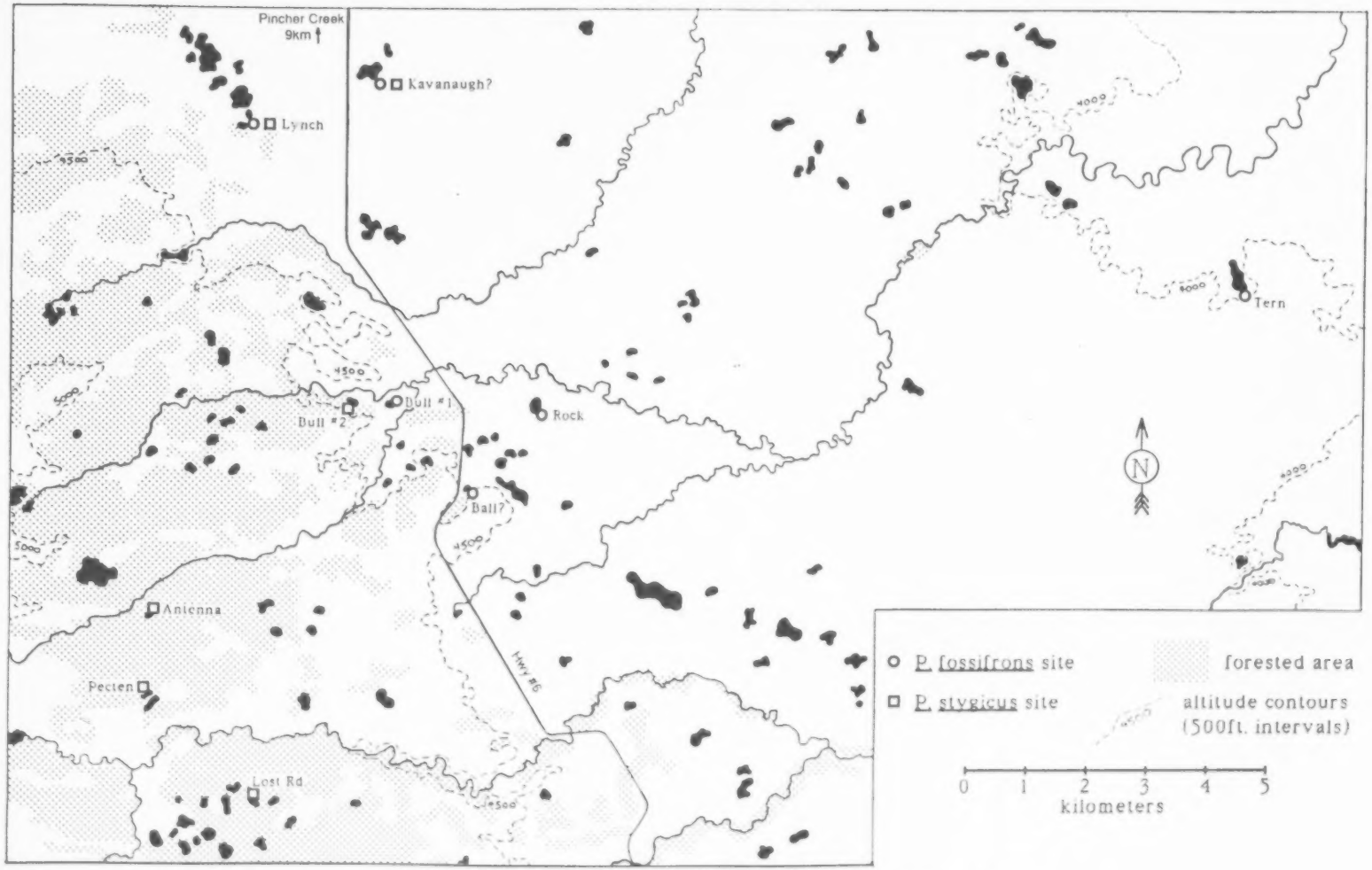


Figure 4.1: Patrobus fossifrons and P. stygicus sites in the Pincher Creek area of southwestern Alberta.

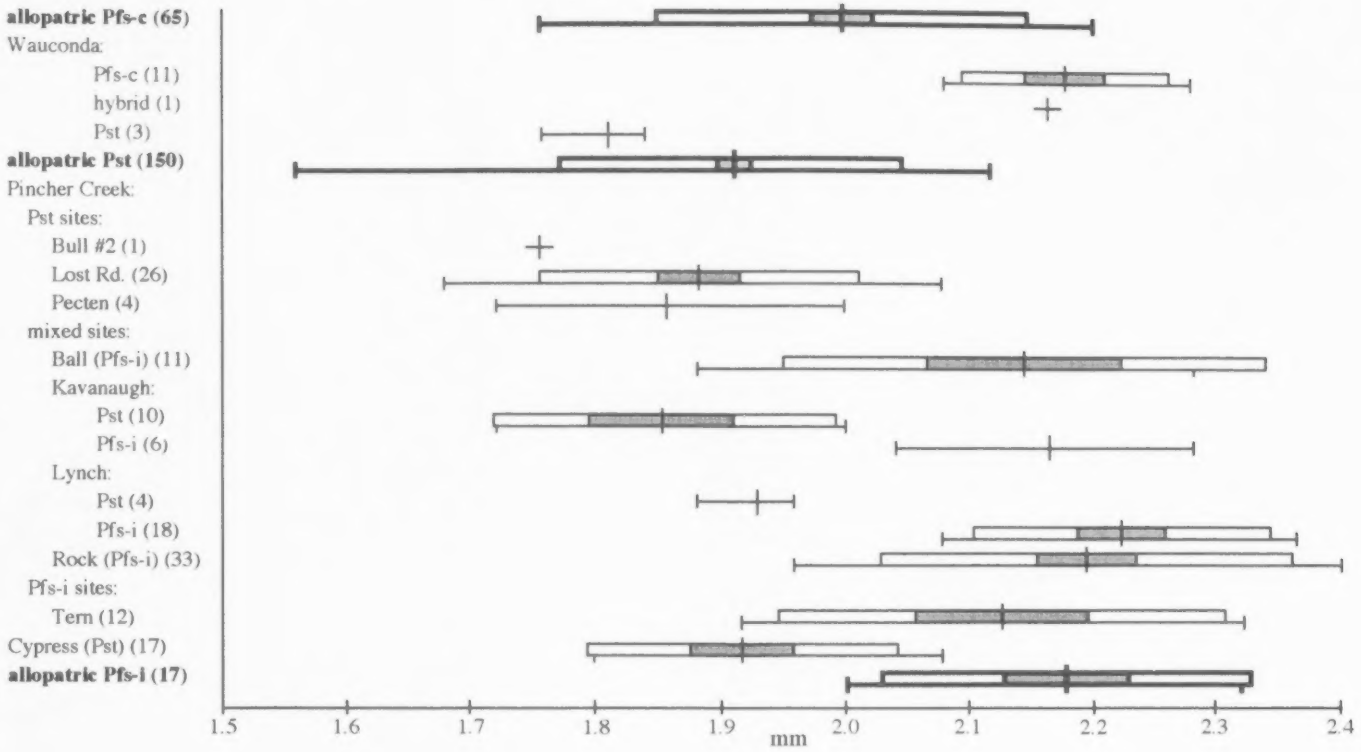


Figure 4.2: Pronotal length of females from sympatric populations. Pfs-c = 'coastal' Patrobus fossifrons; Pfs-i = 'inland' P. fossifrons; Pst = P. stygicus. Allopatric measurements included for comparison are pooled samples discussed in Chapter 3. Sample sizes are in brackets. Based on the procedure of Hubbs and Hubbs (1953)*.

* Horizontal line = range of measurements; vertical line = mean; outlined box = mean ± 1.5 standard deviations; shaded box = mean ± 2 standard errors.

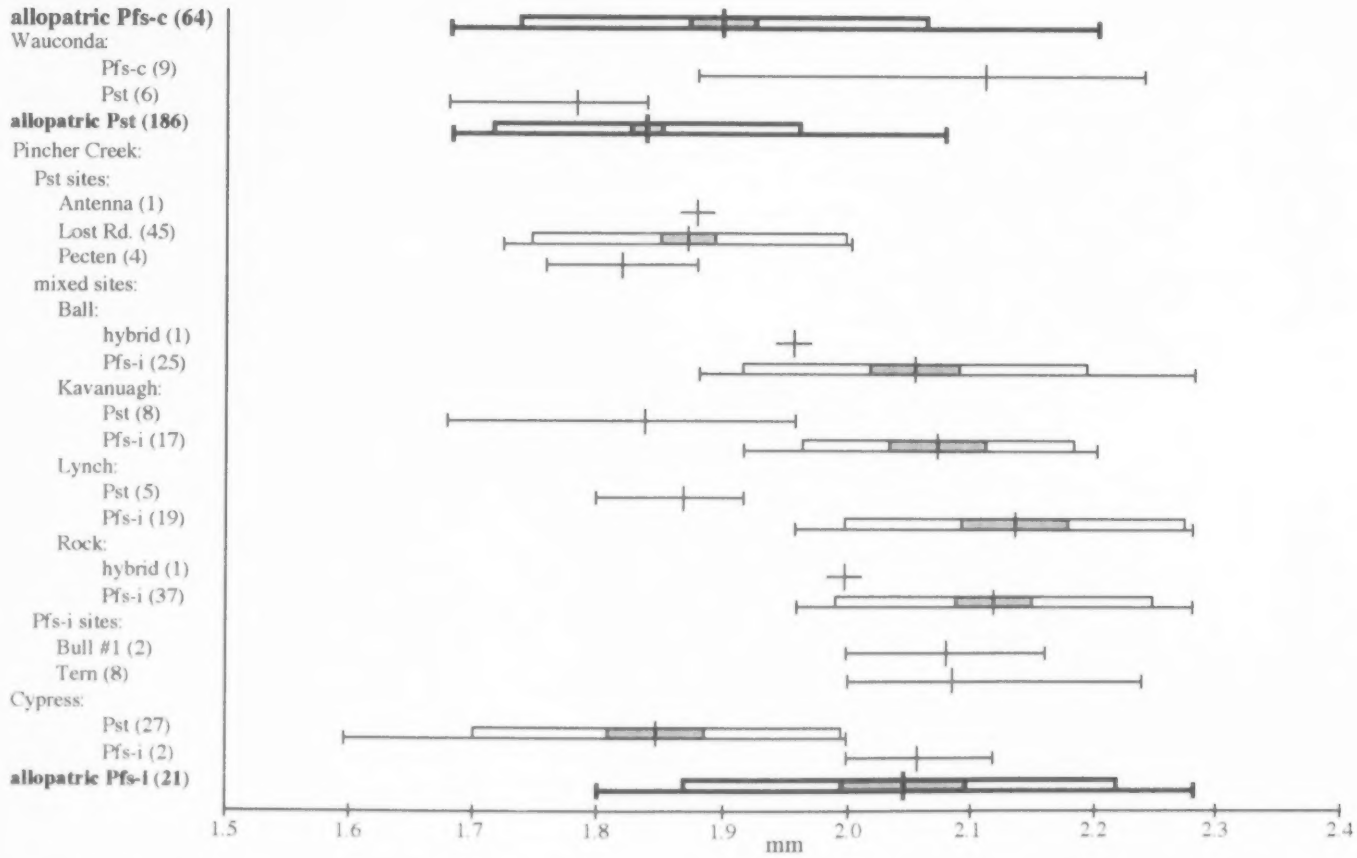


Figure 4.3: Pronotal length in males from sympatric populations. Pfs-c = 'coastal' Patrobus fossifrons; Pfs-i = 'inland' P. fossifrons; Pst = P. stygicus. Allopatric measurements included for comparison are pooled samples discussed in Chapter 3. Sample sizes are in brackets. Based on the procedure of Hubbs and Hubbs (1953)*.

* Horizontal line = range of measurements, vertical line = mean, outlined box = mean ± 1.5 standard deviations, shaded box = mean ± 2 standard errors.

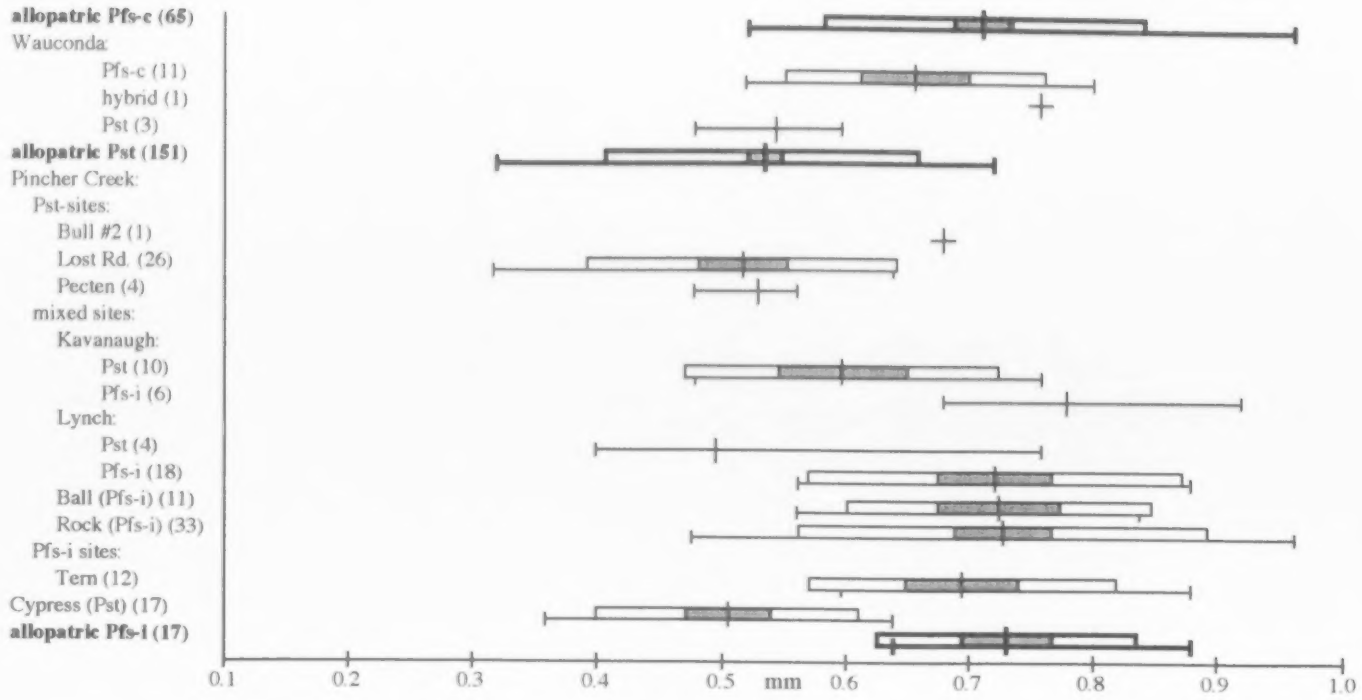


Figure 4.4: Pronotal width minus length in females from sympatric populations. Pfs-c = 'coastal' Patrobus fossifrons; Pfs-i = 'inland' P. fossifrons; Pst = P. stygicus. Allopatric measurements included for comparison are pooled samples discussed in Chapter 3. Sample sizes are in brackets. Based on the procedure of Hubbs and Hubbs (1953)*.

* Horizontal line = range of measurements; vertical line = mean; outlined box = mean ± 1.5 standard deviations; shaded box = mean ± 2 standard errors.

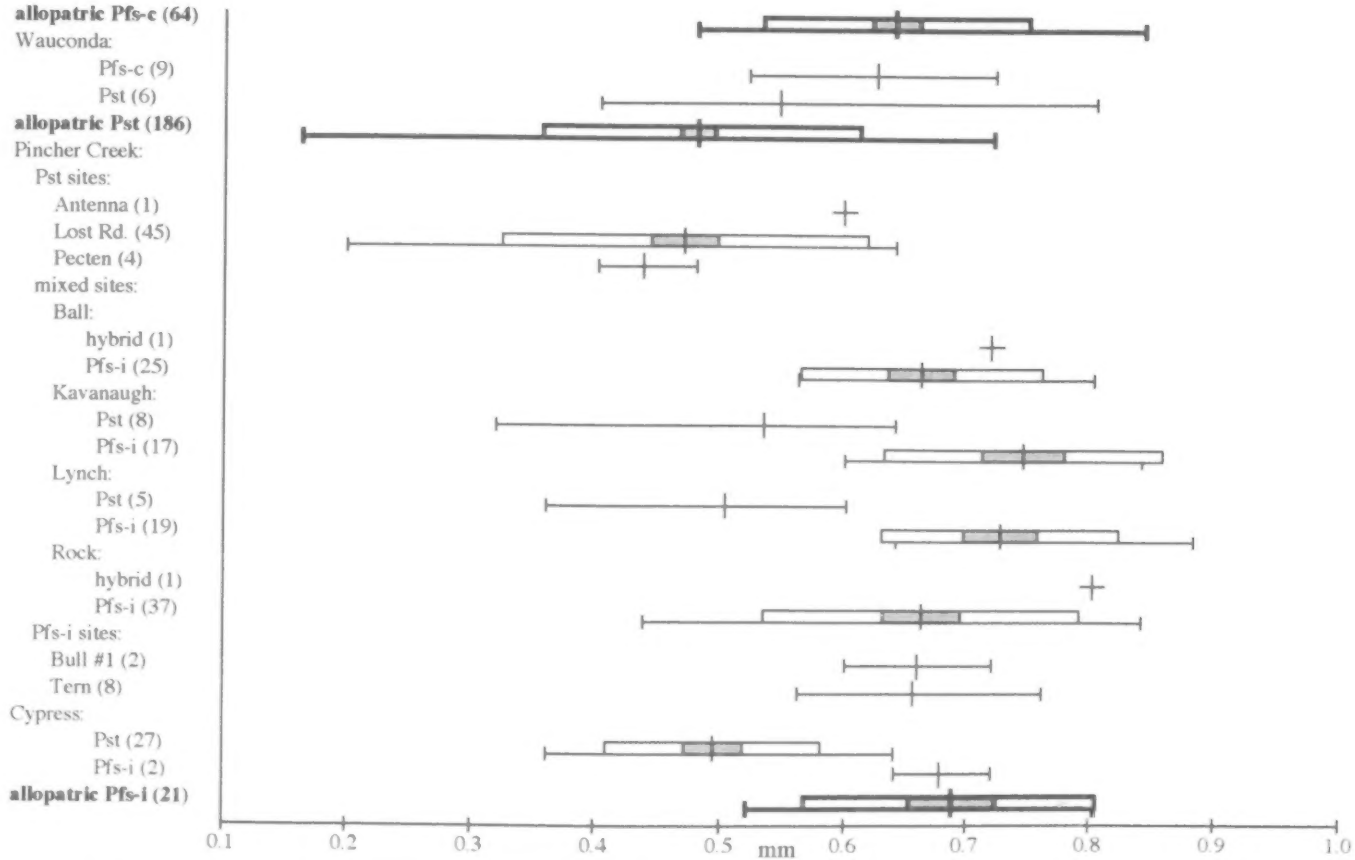


Figure 4.5: Pronotal width minus length in males from sympatric populations. Pfs-c = 'coastal' Patrobus fossifrons; Pfs-i = 'inland' P. fossifrons; Pst = P. stygicus. Allopatric measurements included for comparison are pooled samples discussed in Chapter 3. Sample sizes are in brackets. Based on the procedure of Hubbs and Hubbs (1953)*.

* Horizontal line = range of measurements, vertical line = mean, outlined box = mean ± 1.5 standard deviations, shaded box = mean ± 2 standard errors.

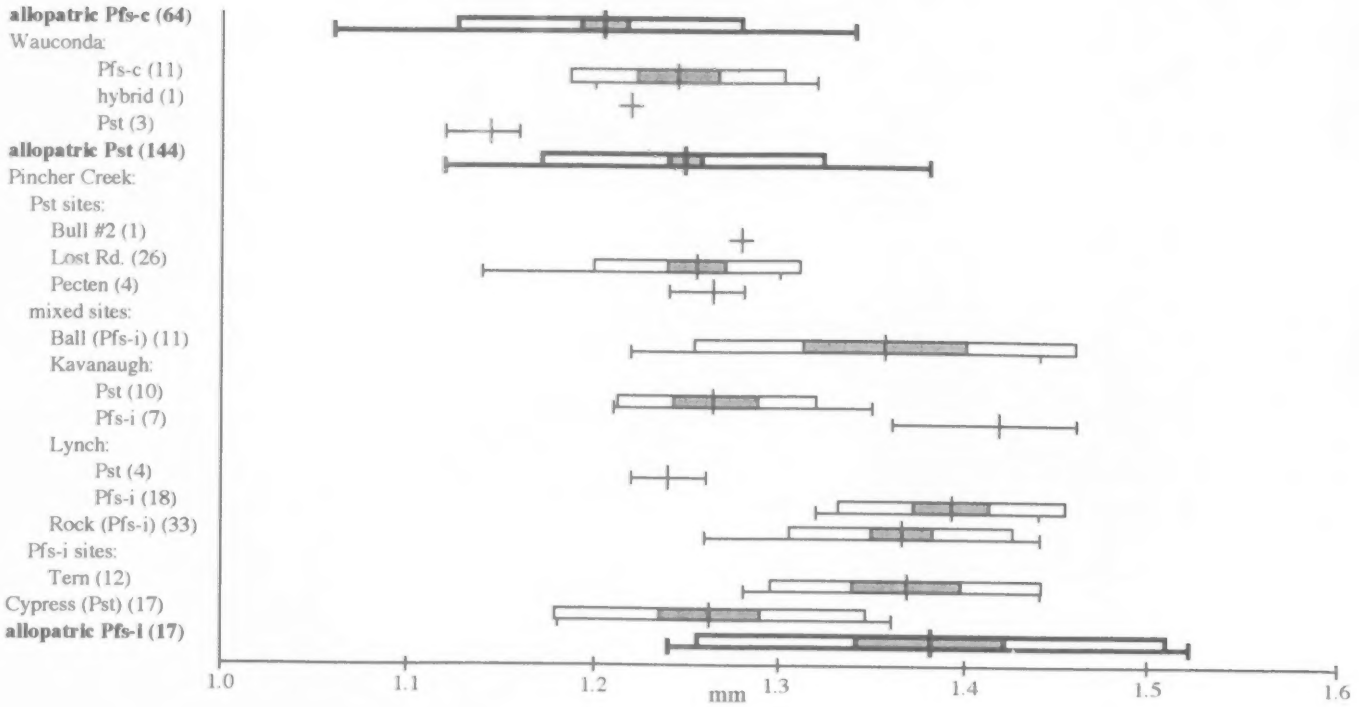


Figure 4.6: Antenna tip length (sum of lengths of antennomeres nine, 10, and 11) of females from sympatric populations. Pfs-c = 'coastal' Patrobus fossifrons; Pfs-i = 'inland' P. fossifrons; Pst = P. stygicus. Allopatric measurements included for comparison are pooled samples discussed in Chapter 3. Sample sizes are in brackets. Based on the procedure of Hubbs and Hubbs (1953)*.

* Horizontal line = range of measurements; vertical line = mean; outlined box = mean ± 1.5 standard deviations; shaded box = mean ± 2 standard errors.

POHL - MSc Thesis

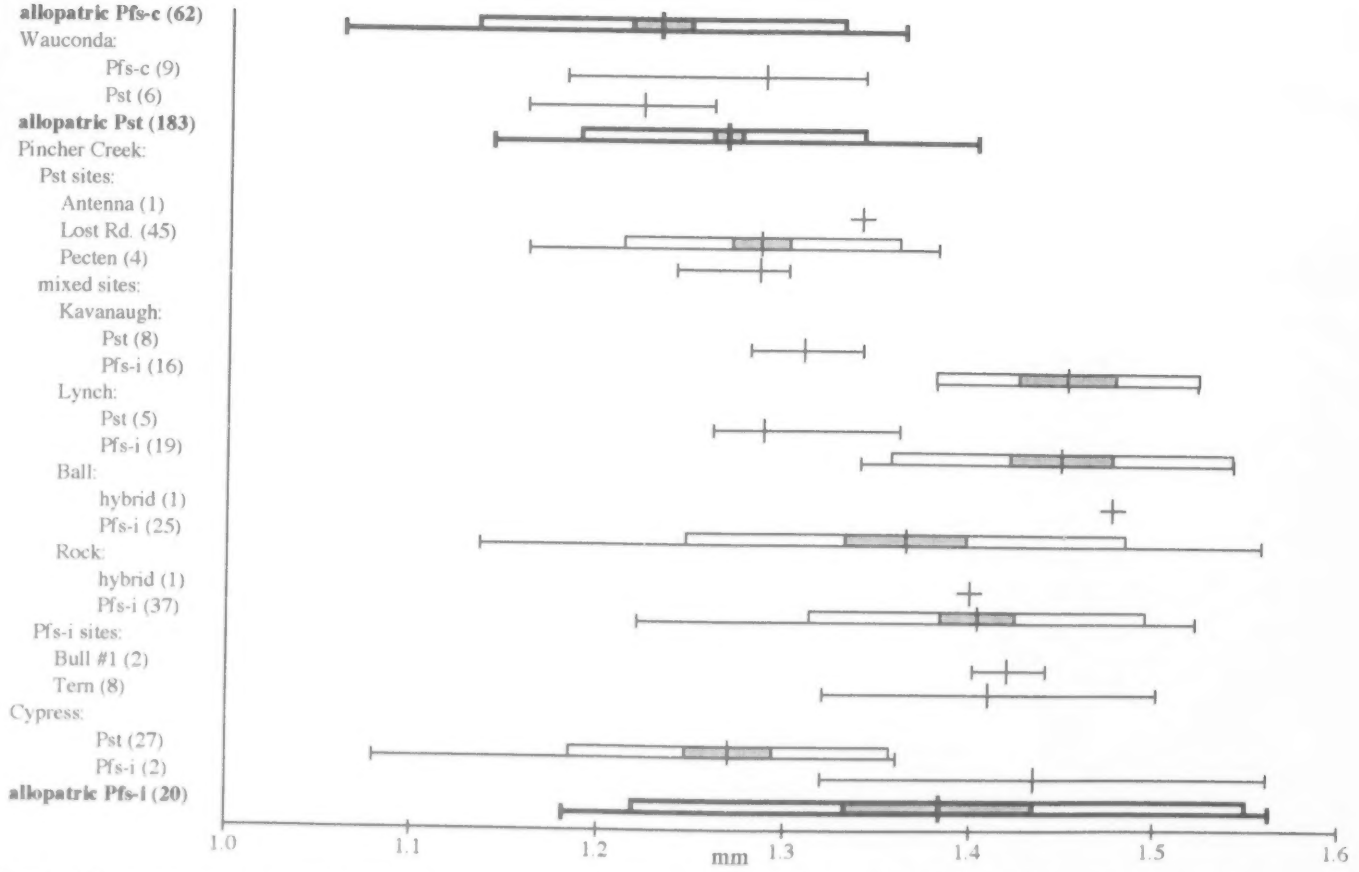


Figure 4.7: Antenna tip length (sum of lengths of antennomeres nine, 10, and 11) of males from sympatric populations. Pfs-c = 'coastal' Patrobus fossifrons; Pfs-i = 'inland' P. fossifrons; Pst = P. stygicus. Allopatric measurements included for comparison are pooled samples discussed in Chapter 3. Sample sizes are in brackets. Based on the procedure of Hubbs and Hubbs (1953)*.

* Horizontal line = range of measurements; vertical line = mean; outlined box = mean ± 1.5 standard deviations; shaded box = mean ± 2 standard errors.

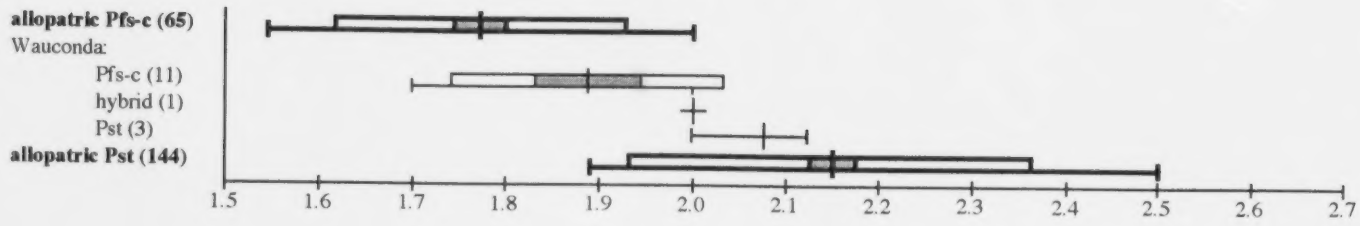


Figure 4.8: Ninth antennomere squareness (length divided by width) of females from sympatric populations. Pfs-c = 'coastal' Patrobus fossifrons; Pfs-i = 'inland' P. fossifrons; Pst = P. stygicus. Allopatric measurements included for comparison are pooled samples discussed in Chapter 3. Sample sizes are in brackets. Based on the procedure of Hubbs and Hubbs (1953)*.

* Horizontal line = range of measurements; vertical line = mean; outlined box = mean ± 1.5 standard deviations; shaded box = mean ± 2 standard errors.

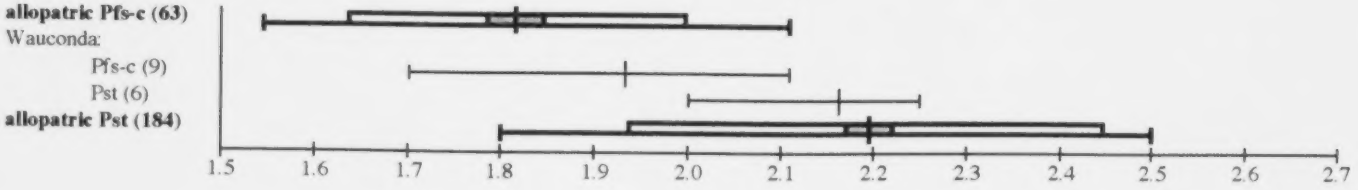


Figure 4.9: Ninth antennomere squareness (length divided by width) of males from sympatric populations. Pfs-c = 'coastal' Patrobus fossifrons; Pfs-i = 'inland' P. fossifrons; Pst = P. stygicus. Allopatric measurements included for comparison are pooled samples discussed in Chapter 3. Sample sizes are in brackets. Based on the procedure of Hubbs and Hubbs (1953)*.

* Horizontal line = range of measurements; vertical line = mean; outlined box = mean ± 1.5 standard deviations; shaded box = mean ± 2 standard errors.

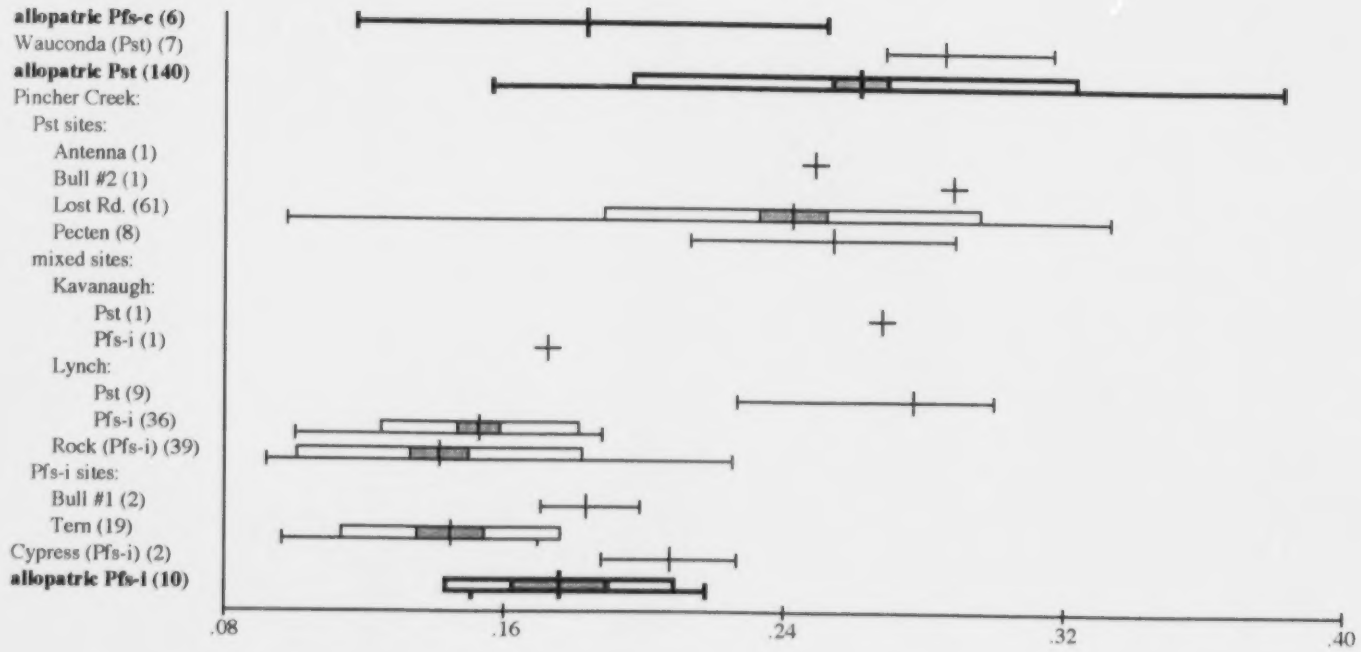


Figure 4.10: Relative length of R_1 wing vein, in sympatric populations. Pfs-c = 'coastal' Patrobus fossifrons; Pfs-i = 'inland' P. fossifrons; Pst = P. stygicus. Allopatric measurements included for comparison are pooled samples discussed in Chapter 3. Sample sizes are in brackets. Based on the procedure of Hubbs and Hubbs (1953)*.

* Horizontal line = range of measurements; vertical line = mean; outlined box = mean ± 1.5 standard deviations; shaded box = mean ± 2 standard errors.

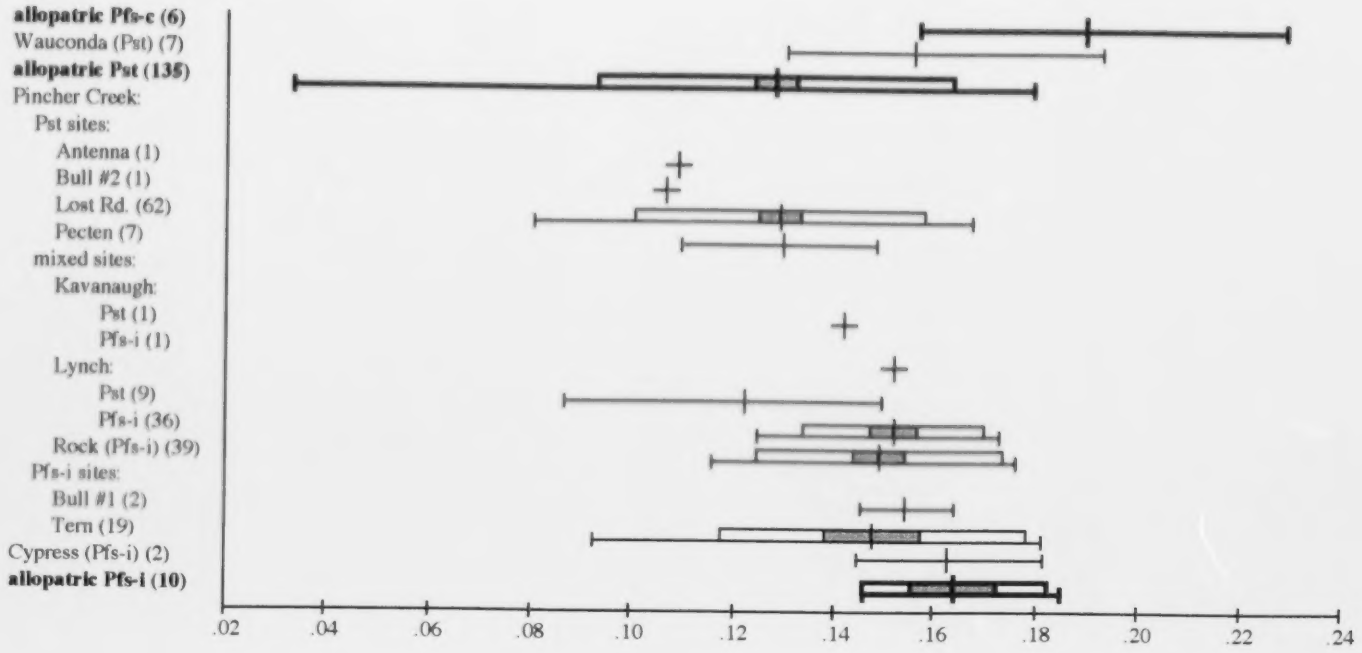


Figure 4.11: Relative width of wing wedge cell, in sympatric populations. Pfs-c = 'coastal' Patrobus fossifrons; Pfs-i = 'inland' P. fossifrons; Pst = P. stygicus. Allopatric measurements included for comparison are pooled samples discussed in Chapter 3. Sample sizes are in brackets. Based on the procedure of Hubbs and Hubbs (1953)*.

* Horizontal line = range of measurements; vertical line = mean; outlined box = mean ± 1.5 standard deviations; shaded box = mean ± 2 standard errors.

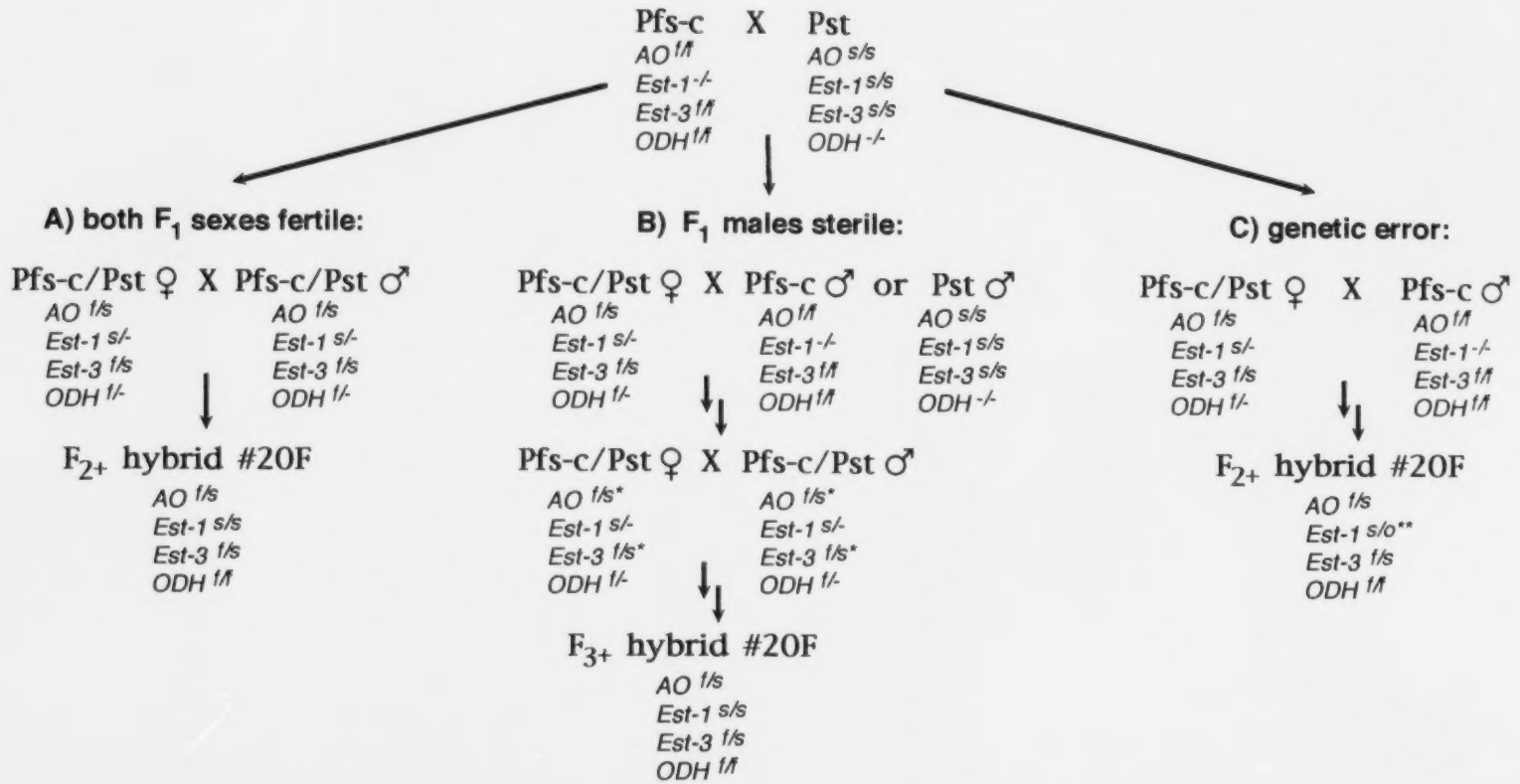


Figure 4.12: Possible ancestries of the Wauconda specimen of mixed 'coastal' Patrobus fossifrons (Pfs-c) / P. stygicus (Pst) stock. A single hybridization event is assumed to have occurred. Double arrows indicate where more than one generation may have occurred. '*f*' indicates Pfs-c alleles Ao^G, $Est\text{-}3^K$, and Odh^F; '*s*' indicates Pst alleles Ao^E, $Est\text{-}1^F$, and $Est\text{-}3^L$. Dashes indicate unspecified alleles.

* These loci could have been *f/f*, *f/s*, or *s/s*. One of these beetles contributed an '*f*', and the other contributed an '*s*' to hybrid specimen #20F, at both *AO* and *Est-3*.

** The '*o*' indicates a null allele where genetic information is purported to have been lost.

5. EVIDENCE AND ESTIMATES OF GENE FLOW

5.1 Theory

5.1.1 Indirect Evidence of Gene Flow

Morphological Evidence of Gene Flow

If gene flow is occurring between two taxa, then members of sympatric populations of those taxa ought to be morphologically more similar than are members of allopatric populations. Discriminant function analysis, developed by Fisher (1936), is a method of comparing two populations by examining several characters simultaneously. It combines a set of variables into a single function, which maximizes the difference between two samples. The resulting discriminant function is a summary of the variation expressed in a series of variables. This procedure can be used to search for gene flow between two taxa, by comparing distances in allopatry to differences in sympatry. It can also be used to examine hybrids, by plotting them on the discriminant function to see where they lie with respect to the parent species.

In the current study, if gene flow has been occurring, a discriminant function analysis of morphological characters ought to show less difference between sympatric populations of 'coastal' and 'inland' P. fossifrons (Pfs-c and Pfs-i, respectively), and P. stygicus (Pst) than between allopatric populations. As well, the probable hybrids ought to be between the parent species on the discriminant function plot, if they are indeed hybrids.

Electrophoretic Evidence of Gene Flow

If gene flow is occurring between Pfs and Pst, they ought to be electrophoretically more similar in sympatry than they are in allopatry. Nei's (1978) genetic identities are useful for this sort of comparison. If sympatric populations of two different taxa have a higher identity value than allopatric ones, it is an indication that they are interbreeding. See Chapter 3 for Nei's formula for computing these distances.

Heterozygosity and Bilateral Symmetry Evidence of Gene Flow

Heterozygosity is thought to affect the fitness of organisms, and bilateral symmetry provides a useful measure of that fitness; see Palmer and Strobeck (1986) for a review. Generally, the more diverse a populations' gene pool is, the more evolutionarily fit the population ought to be (Lerner 1954), because a variety of alleles at a locus provides several biochemical, structural, or behavioral responses to deal with environmental factors.

Asymmetry of otherwise bilaterally symmetrical organisms is an indication of deviation from the ideal developmental blueprint. More heterozygous populations have been shown to be more symmetrical in insects (Biémont 1983), molluscs (Kat 1982), and fish (Leary *et al.* 1985a).

According to Palmer and Strobeck (1986) the bilateral variance is the most informative measure of symmetry. It is calculated as follows:

[5.1] $$V = \frac{\sum(R-L)^2}{N}$$

where **V** is the measure of bilateral variance, **N** is the sample size, and **R** and **L** are the left and right measures, respectively. This is slightly different from the true variance, in that zero, rather than the mean of the R and L measures, is considered to be the center point about which the measurements are distributed. It is still a measure of variance, and as such, it can be analysed statistically.

Heterozygosity is also thought to affect symmetry at the individual level. More heterozygous individuals are genetically more fit, so they ought to show higher levels of symmetry than more homozygous individuals. This has been shown in fish (Leary *et al.* 1983, 1984).

Though heterozygosity generally results in increased symmetry, this is not so when it involves the incorporation of genetic information which is too different. Interspecific differences in DNA are often too great, so alleles can be deleted, or can fail to function properly when different species hybridize. As a result, many inter-species hybrids, though highly heterozygous, show decreased symmetry (Graham and Felley 1985, Leary *et al.* 1985b). Therefore, the relationship between heterozygosity and symmetry can be informative. One would expect that, in allopatric populations, more heterozygous individuals should be more symmetrical. In sympatric populations, if symmetry decreases as heterozygosity increases, this is a sign that gene flow is occurring.

In the current study, if gene flow is occurring, sympatric Pfs and Pst ought to be more heterozygous and less symmetrical than allopatric populations. As well, if heterozygosity increases as symmetry decreases at sympatric sites, then gene flow is likely causing some of the heterozygosity.

5.1.2 Electrophoretic-Based Estimates of Gene Flow

In addition to indirect evidence of gene flow, there are techniques for estimating actual levels of gene flow. It is usually measured as **Nm**, where **N** is the population size, and **m** is the fraction of individuals in the population which are migrants. The most common calculation of Nm is based on Wright's (1951) correlation coefficient, $\mathbf{F_{ST}}$. This is essentially a fixation index of subpopulations within a population, based on differences in the levels of homozygosity within and between subpopulations. For completely undifferentiated subpopulations, F_{ST} is zero, and for completely differentiated subpopulations, it is one. To estimate Nm from F_{ST}, Wright developed the formula:

[5.2] $$Nm_{est} = \frac{(1/F_{ST}) - 1}{4}$$

Alleles are assumed to be neutral to selective forces by this model, so if they are not, biased estimations of Nm will result. This formula works for Slatkin's (1985b) "infinite island" model of population structure. It also works on his "n-island" model, if the number of islands (subpopulations) is large, and the mutation rate of alleles is small. If the population structure conforms to the "stepping-stone" model of Kimura (1953), then this calculation will underestimate Nm. For most applications, it gives a reasonable estimate.

Another method of estimating Nm, also based on differences in homozygosity, was described by Nei (1975), in which he used his D-value (Nei 1972, 1978) to estimate Nm. This method is based on the principle that the genetic distance between populations decreases as the gene flow between them increases. Like the F_{ST} calculations, this estimate depends on differences in homozygosity, since that is what Nei's D measures are based on. To estimate Nm, one first estimates m as follows:

[5.3] $$EXP(-D) = \frac{m}{m+\mu}$$

where **D** is the genetic distance between the populations in question, **m** is the fraction of the populations which migrate, and $\boldsymbol{\mu}$ is the mutation rate of the alleles used in the analysis (assumed to be constant at $2X10^{-6}$). One then estimates m, which can be left as is, or a value of N can be determined or assumed, to arrive at Nm. This equation only works for an "n-island" model of population structure, and when mutation and selection rates are very small.

Nei's technique works in theory, but as Slatkin (1985b) points out, it is subject to error. If the average selection coefficient is as large as or larger than m, biased estimates will result. Also, it requires a reasonably accurate measure of N, if it is to be compared to other Nm values. In light of these problems, the F_{ST} technique, also based on homozygosity differences, is a more reliable estimator of Nm.

A third technique for estimating Nm was developed by Slatkin (1985a). This method is based on private alleles; alleles which are present in only one subpopulation. Generally, the more gene flow there is between subpopulations, the lower the average frequency of private alleles, $\bar{\mathbf{P}}_1$. When a large amount of gene flow is taking place, private alleles are highly unlikely to remain private unless they are present only at low frequencies. Slatkin found that, when he plotted Nm against $\bar{P}_1$, he got a more or less linear relationship:

[5.4] $$\ln\bar{P}_1 = a \cdot \ln(Nm) + b$$

where **a** = -0.505, and **b** = -2.440. Thus his formula for estimating Nm is as follows:

[5.5] $$Nm_{est} = EXP\left[\frac{\ln(\bar{P}_1)+2.440}{-0.505}\right]$$

This relationship is more or less linear for values of Nm between 0.01 and 10. (Lower values tend to be overestimated, and higher values tend to be underestimated.) This method yields correct results under different population structures, but it is prone to error if mutations are not selectively neutral. It is designed for a sample size of 25, but can be corrected by multiplying the resultant Nm estimate by 25/N, where N is the sample size.

In intra-taxon comparisons, a fundamental problem arises with Slatkin's technique, because more than two populations are being compared simultaneously. In a situation where there are two groups of populations interbreeding among themselves, but not with each other, there would be very few private alleles. Most alleles would be shared by other populations within the group, though not with populations in the other group. A calculation of Nm based on the frequency of private alleles would thus vastly overestimate gene flow. This can be avoided by pooling populations into their respective subgroups. Another solution is to calculate Nm between all possible pairs of populations. An unpublished subroutine for Swofford and Selander's (1981) BIOSYS-1 program, written by B. M. Rolseth, does this.

Wright's technique, based on allelic fixation levels within populations, detects this subgrouping. However, Wright's method cannot determine which subpopulations are

related. The best approach in situations such as this is to use Wright's technique to estimate overall levels of gene flow, and then compare pairwise Slatkin's Nm estimates to identify any partitioning into subpopulations.

5.2 Materials and Methods

5.2.1 Discriminant Function Analysis Methods

The discriminant function analysis was carried out with the assistance of C. P. Klingenberg, using the SAS© statistical program (version 6.06.01; ©1989 by SAS Institute Inc., Cary, NC, USA) on the University of Alberta mainframe computer.

In discriminant function analysis, all variables must be of the same type, so only the quantitative measurements were used. Wing measurements were deleted from the analysis, since many specimens were brachypterous, or were not measured for wing characters. This left four measurements; pronotal length (PLN), pronotal width minus length (PWL), antennal segment length (ALN), and antennal segment squareness (ASQ).

All measurements must have similar standard errors, for discriminant function analysis to provide valid results. Standard errors of the above four measurements were compared using a t-test. The standard error of ASQ was found to be larger than those of the other measurements, so it was converted to antennal segment width (AWD), which had a standard error much closer to those of the other measurements.

Using PLN, PWL, ALN, and AWD, a discriminant function was calculated between allopatric Pfs-c and Pst. Then coordinates were calculated for sympatric specimens of these taxa, using the same function. The entire procedure was repeated between Pfs-i and Pst.

5.2.2 Nei's Genetic Identity Calculation Methods

Allopatric populations of the species and morphs were pooled, and Nei's (1978) unbiased genetic identities were calculated between these and sympatric populations. All populations with sample sizes of at least five were used. For the Wauconda population samples, values were calculated both with and without the hybrid specimen. For calculations including the hybrid, two series of genetic identities were calculated; one with the hybrid placed in Pfs-c, and the other with the hybrid placed in Pst. The hybrid was thus shared between the Pfs-c and Pst samples at Wauconda.

5.2.3 Heterozygosity and Symmetry Analysis Methods

Heterozygosities were determined by direct count, from the electrophoretic data (Appendix #4). Heterozygosities of the Wauconda samples including the hybrid specimen were calculated as the average of values with the hybrid in the Pfs sample, and in the Pst sample. This effectively splits the specimen between Pfs-c and Pst, as in previous calculations.

Four wing measurements were used in the symmetry calculations; the length of the sclerotized area of the fused costa, subcosta, and radius (*a* in Figure 2.8), the length of the R_1 vein extending from this sclerotized area (*b* - *a* in Figure 2.8), the length of the cubital vein between the two cubito-anal cross veins (*d* in Figure 2.8), and the width of the wedge cell (*c* in Figure 2.8). These are hereby abbreviated as R1.1, R1.2, W1, and W2, respectively. These measurements, or the measurements they were calculated from, appear in the morphological data, Appendix #5.

Individual symmetries were calculated for each specimen, based on the absolute value of the right wing measure minus the left wing measure, in micrometer units at 25X magnification (1 unit @25X = 40μm). Measurement errors were estimated by measuring a sample of 16 Rock Pond Pfs-i males four times. Percent error in measurement of each character was then calculated for each specimen, and averaged over all individuals (Table 5.1).

Measurements from Rock Pfs-i and Chain Pst, the two largest samples of their respective taxa, were compared to the left-right averages and to other measurements to test for independence. Pearson correlations were calculated between the symmetries and left-right averages, and between the symmetries and PLN, an indicator of beetle size. The only correlation greater than 0.5 was between the symmetry and left-right average of character R1.2, in Rock Pond males. (correlation = 0.746; N = 16). Dividing the symmetry by the left-right average did not reduce the correlation very much (correlation of symmetry/average vs. average = 0.659; N = 16). This fact, and the fact that no correlation was seen in Rock females, or in Chain Pond beetles, suggest that it was an anomaly, and not of biological significance. It was concluded that the measures of symmetry were generally independent of the left-right averages of the measures, and independent of overall beetle size.

Bilateral variances of were calculated from individual symmetries using Palmer and Strobeck's (1986) formula (see Section 5.1.3). These symmetries were compared to error variances for a sample of 16 Rock Pond Pfs-i males, to test for significance of bilateral variances (Table 5.1). Bilateral variances were found to be significantly larger than

measurement variances for all four measurements in this sample, so this is assumed to be so in other samples as well.

Bilateral variances of males and females were then examined for sexual differences, in each population sample. Only one of the 20 tests was significant at a 95% confidence interval (symmetry of W1 in Lynch Pfs-i; F = 3.940; degrees of freedom = 15, 18). On average, one in 20 tests ought to be falsely considered significant, because the test is only accurate 95% of the time. Thus this one significant difference is considered to be testing error, and not of biological significance. As well, the fact that no sexual differences were found in the characters R1 and W (see Chapter 2 for details), indicates that variability in wing characteristics is not sex dependent in Patrobus. There could be differences in the way heterozygosity affects the sexes, but splitting samples into males and females would result in such small samples that any significant differences of this nature probably could not be detected. It is concluded that the bilateral variances are independent of other measures, and independent of sex. In all further comparisons of symmetry, data from both sexes were pooled together.

Tests for significant differences between the bilateral variances of different population samples were done using Sokal and Rohlf's (1981) test for significance of differences between two variances.

All populations for which complete morphological and genetic data was available for at least 20 specimens, were used in the comparison of heterozygosity and asymmetry. Specimens with incomplete data were also used, in those tests for which their data were complete (i.e. populations with missing electrophoretic data were used in some of the tests of individual loci). No Pfs-c populations were analysed, because not enough individuals were macropterous.

5.2.4 Gene Flow Estimation Methods

Using the electrophoretic data, Nm values were estimated via Wrights' and Slatkin's methods. All of Slatkin's estimates were corrected for sample size. Estimates of m were also calculated using Nei's unbiased D, which corrects for small sample sizes. This cannot be calculated when more than two populations are being compared simultaneously, unless some of them are pooled, so some m estimates could not be made.

Amounts of gene flow were estimated between Pfs and Pst in allopatry and in sympatry. At Pincher Creek, gene flow was estimated at the Lynch Lakes sympatric site, and between locally allopatric populations of both species at other sites in the sympatric zone. At Wauconda, estimates of gene flow between Pfs-c and Pst were calculated without

the hybrid specimen, and with the hybrid specimen split between the two taxa, as in the Nei's identity calculations (see Section 5.2.2 for details).

The homogeneity of Pst and of Pfs was tested by estimating gene flow within these species. In the latter tests, besides Nm estimates between all populations simultaneously, pairwise Slatkin's calculations between intraspecific populations were made, to test for subgrouping of populations within species.

5.3 Results

5.3.1 Morphological Introgression as Evidence of Gene Flow

Hubbs-Hubbs plots of eigenvalues resulting from the discriminant function analysis appear in Figures 5.1 and 5.2. Figure 5.1, a comparison of Pfs-i to Pst, shows that, generally, the sympatric populations conformed to the characteristics of their respective species in allopatry. However, the range of female Pincher Creek Pfs-i measurements extends well into the range of measurements for Pst. A re-examination of the Hubbs-Hubbs plots of individual characters (Figures 4.2 to 4.11) shows that there were several outliers in the Pincher Creek Pfs-i population samples. For example, Rock Pond Pfs-i values were somewhat extended into the range of Pst measures for PWL and ALN, and Ball's Pond Pfs-i were extended for PLN, PWL, and ALN. Several Ball's Pond males also had slightly *stygicus-like* median lobes. Various analyses of variance and skewness were done (not presented here) to determine whether this skewing of Pfs-i character states in the direction of Pst character states was significant. Results were inconclusive, due to the sizes of samples analyzed. However, it is possible that female Pfs-i from the Pincher Creek area are slightly more *stygicus-like* than their allopatric counterparts. If this is really the case, it would be an indicator of gene flow between the species there.

Figure 5.2 compares Pfs-c and Pst. The Wauconda Pfs-c females were significantly more *stygicus-like* than allopatric Pfs-c. As well, both male and female Pst at Wauconda appeared to be more *fossifrons-like*, but samples were not large enough to determine this with certainty.

Figures 5.1 and 5.2 also show that the putative hybrid specimens all lie well within the range of P. fossifrons measurements. However, the multivariate analysis did not take into account some of the characters which were intermediate in these specimens.

In summary, some evidence indicates that morphological Pst characters have introgressed into a few Pfs-i females at Pincher Creek. At Wauconda, introgression of foreign characters into both Pfs-c and Pst appears to be more widespread .

5.3.2 Electrophoretic Introgression as Evidence of Gene Flow

Table 5.2 lists Nei's (1978) unbiased genetic identities and distances for sympatric and pooled allopatric populations.

The sympatric Pfs-i population samples from Tern, Rock, and Lynch were not significantly closer to the various population samples of Pst than allopatric Pfs-i were. However, the Pst samples exhibited differences when compared to the Pfs-i samples as a whole. Lost Rd. specimens were no closer to Pfs-i than allopatric Pst were, but Lynch Pst were closer to Pfs-i. (Pecten specimens were also slightly closer to Pfs-i, but this was not considered to be significant in light of the small size of this sample). This indicates that gene flow may have occurred into Pst at Lynch Lakes.

Pfs-c from Wauconda were closer to Pst than were Pfs-i. Unfortunately, no large sample of allopatric Pfs-c were electrophoresed, so I do not know whether Pfs-c were any closer to Pst in sympatry than in allopatry. Wauconda Pst specimens, including the hybrid, were much closer than allopatric Pst were to Wauconda Pfs-c. This was partly due to the hybrid, but when it was removed, the remaining Wauconda Pst were still somewhat closer to Pfs-c than allopatric Pst were.

In summary, Nei's (1978) unbiased identities suggest that gene flow has not occurred into any Pfs-i populations. Pfs-c could not be examined in this manner, due to lack of data. Nei's identities also suggest that gene flow has occurred into Pst populations at Lynch Lakes and at Wauconda.

5.3.3 Heterozygosity and Symmetry

Heterozygosities

Direct count heterozygosities appear in Table 5.3. Independent t-tests for significant differences in heterozygosity between sympatric and allopatric populations also appear in this table. Variances were kept separate in these tests. Sympatric Pfs-c and Pst populations did not show any significant differences from pooled allopatric populations. For Pfs-i populations, the only significant difference was that the Tern sample had a significantly lower heterozygosity than allopatric Pfs-i. It is concluded that, in sympatry, heterozygosities were not any larger than in allopatry.

Symmetries

Table 5.4 shows bilateral variances of the study populations. Statistical tests were done between all possible pairs of populations (Table 5.5). The only trend is that Chain Pst were highly asymmetrical. Since it is an allopatric population, this cannot be due to gene flow from Pfs-i. Heterozygosity of the Chain Pst population was not the highest nor the lowest of the allopatric Pst, so the high level of asymmetry in Chain Pst was probably not caused by unusually high or low heterozygosity. The sympatric populations (Rock and Lynch Pfs-i, and Lost Rd. Pst) were no less symmetrical than pooled allopatric populations, so this test provides no evidence of gene flow in sympatry.

Heterozygosity versus Symmetry

Plots of heterozygosity versus symmetry appear in Figure 5.3. No clear pattern is evident for Lynch Pfs-i, Chain Pst, or George Pst. However, Rock Pfs-i and Lost Rd. Pst seem to decrease in symmetry as heterozygosity increases. Due to the small sizes of some heterozygosity classes, statistical tests of symmetry measures in these populations are not very informative. However, some ANOVA tests were carried out on measurements which showed consistent trends. Significant differences between heterozygosity classes, at a 95% confidence interval, were as follows. Rock Pond individuals with heterozygosity of 0.375 were significantly less symmetrical for R1.2 than individuals with lower heterozygosities ($F = 5.446$, degrees of freedom = 3, 25). Also, Lost Rd. Pond individuals with higher heterozygosities (0.250 and 0.375) were less symmetrical for R1.1 and R1.2 than individuals with lower heterozygosities (0 and 0.125) (R1.1 $F = 3.985$, degrees of freedom = 4, 31; R1.2 $F = 3.379$, degrees of freedom = 4, 29). This is evidence that, in sympatry, increased heterozygosity sometimes leads to decreased symmetry. However, Lynch Pfs-i were also in the sympatric area, but they did not show any significant decrease in symmetry as heterozygosity increased.

The previous test showed that different measures responded differently to heterozygosity level. To test the hypothesis that symmetry in these measures is also dependent on which loci are heterozygous, specimens in each population were grouped depending on whether they were homozygous or heterozygous, locus by locus. Too few heterozygous individuals were present at *Ao* or *Apk*, so those loci are deleted from the analysis. Bilateral variances of the above groupings were then compared (Table 5.6). This table also includes details of F-tests between homozygous and heterozygous individuals in each population, for each measure of bilateral variance. In a few cases, heterozygous individuals were less symmetrical than homozygous individuals, but generally very few differences were found. Of the differences which were found, all but two depended on

heterozygosity levels in the *Est-3* locus. Rock Pond Pfs-i showed the most differences, including the two which did not involve *Est-3*.

The dependence of symmetry on heterozygosity at *Est-3* suggests that, whether or not it is in allopatric populations, *Est-3* heterozygosity decreases developmental homeostasis. This is the opposite to what has been reported in other studies (see Section 5.1.3).

Rock Pond showed significantly lower symmetry in heterozygous individuals, at three different loci. Clearly, the more heterozygous individuals in this population are disadvantaged developmentally. This suggests that foreign genes are introgressing into Rock Pond Pfs-i, to cause this disadvantage.

In summary, sympatric populations of Pfs and Pst were no more heterozygous, nor any less symmetrical than their allopatric counterparts. However, the sympatric area populations of Rock (Pfs-i) and Lost Rd. (Pst) showed a decrease in symmetry with overall heterozygosity. When heterozygosity at specific loci was compared to symmetry levels, Rock specimens showed a decrease in symmetry with heterozygosity at three loci. These facts provide some evidence that sympatric area populations were negatively affected by increased heterozygosity. This may be a result of introgression of foreign genes into sympatric sites, but the evidence is far from conclusive.

Both allopatric and sympatric populations showed that symmetry decreases with heterozygosity at the *Est-3* locus, suggesting that *Est-3* affects, or is closely linked to a locus which affects developmental homeostasis. As well, the allopatric Chain Pond Pst beetles were less symmetrical than other populations, though they were no more or less heterozygous.

5.3.4 Estimates of Gene Flow

A summary of gene flow estimates appears in Table 5.7. Details of some F_{ST} calculations appear in Table 5.8. Pairwise Slatkin's Nm estimates appear in Tables 5.9 and 5.10. Although Nei's estimates are useful to show relative levels of gene flow, they cannot show absolute levels without a realistic estimate of population size. They are included in Table 5.7, but further discussion will be restricted to Wright's and Slatkin's Nm estimates.

The lowest Slatkin's Nm estimate, 0.009, is just outside the range of accurate estimation (0.01 to 10), so it may be slightly overestimated. As well, some of the Nm estimates within Pst, and one of the estimates within Pfs-c, are greater than 10, so they may be underestimated. The other Slatkin's estimates are within this range, so they provide useful estimates of gene flow.

Population size is probably the reason for differences between Slatkin's and Wright's Nm estimates. Slatkin's estimate treats the sample as the population, rather than as a subset of the population. It is corrected for the actual sample sizes; resulting Nm values are estimates of how many organisms in the sample are migrants. Wright's Nm, on the other hand, is an estimate of gene flow in the population from which the sample came. His estimate does not actually require a value for N, but the theory behind his technique is based on the assumption of a reasonably large population. Thus, Wright's Nm estimates in the current study are almost an order of magnitude larger than Slatkin's. These values of Nm, then, are not entirely accurate. However, the trends they exhibit, which are the same by either measure, are much more useful.

Gene Flow Estimates Between Pfs-i and Pst

Both Wright's and Slatkin's estimates show that gene flow between allopatric Pfs-i and Pst was very low. It probably was not any higher between locally allopatric populations in the sympatric area, such as Tern Pond (Pfs-i), and Lost Rd. and Pecten Ponds (Pst). However, gene flow did appear to be slightly higher at Lynch Lakes, the sympatric site in the Pincher Creek area.

With Wright's F_{ST} values, one can examine individual loci and even alleles to see which ones are causing changes in Wright's Nm estimates (Table 5.8). At Lynch Lakes, Wright's Nm was slightly higher due to a decrease in the fixation levels of electromorphs. The alleles in question are ones which were not shared by the two species. This means that Pfs-i and Pst were not really any closer to each other at Lynch Lakes; they were simply less fixed for their respective alleles.

Allele frequencies of Pincher Creek area populations (Table 2.5) show directly which changes in frequency affected $\bar{P}_1$ and thus Slatkin's Nm. Slatkin's Nm estimate seems to be higher at Lynch Lakes because of slight changes in private allele frequency, and because of fewer private alleles. If more gene flow were going on here, one would expect that alleles which were private in allopatry, would be shared at Lynch Lakes. This is not the case; the private alleles present in Pfs-i and Pst in allopatry, were simply not present in either species at Lynch Lakes. Table 4.2 shows that allopatric Pfs-i and Pst shared five alleles, while Lynch Pfs-i and Pst shared only three. Therefore, the increase in Slatkin's Nm at Lynch Lakes is due to a lack of these alleles, or to sampling error missing the private alleles, not an increase in gene flow. Thus, the higher gene flow at Lynch Lakes, as estimated by Wright's and Slatkin's measures of Nm, is not biologically significant.

Gene Flow Estimates Between Pfs-i and Pst

Gene flow levels between Pfs-c and Pst were somewhat higher than between Pfs-i and Pst. At Wauconda, the sympatric site, Nm was higher than in allopatry, when the hybrid specimen was taken into account. For these interactions, one can also examine frequencies to find the source of this increased gene flow. According to Table 4.2, allopatric Pfs-c and Pst shared six alleles, while in sympatry they shared 12. This was due to the hybrid specimen, which was split between the two samples for this analysis. However, details of Wright's Nm calculations (Table 5.8) show that the higher Nm for Wauconda (as compared to allopatric populations) was due to a more pervasive shift in the entire populations of Pfs-c and Pst. Allele frequencies in Pfs-c and Pst at Wauconda, not including the hybrid, (Table 2.5) have shifted at *Pgi*, to become more similar. Thus, the increased Nm at Wauconda appears to be significant, though the small sample sizes demand a degree of caution.

Gene Flow Estimates Within Species

Table 5.7 also contains gene flow estimates between Pfs-i and Pfs-c. However, samples of both morphs were very small, so the figures are questionable. The sympatric population samples are likely better examples of Pfs than the small allopatric samples. Therefore, calculations were made with all population samples of these two morphs, both sympatric and allopatric (excluding the hybrid specimen). This is probably a more accurate estimate of gene flow between these morphs.

Slatkin's pairwise comparisons of these Pfs populations (Table 5.9) show what previous analysis has already shown; there are clearly two morphs of Pfs, which are quite isolated from each other. These estimates also show that allopatric and Wauconda Pfs-c were not very close genetically. This once again indicates the uncertainty of the genetic makeup of Pfs-c, which was discussed in Chapter 3.

An estimate of gene flow within Pst also appears in Table 5.7. For reasons discussed in the theory section of this chapter, Slatkin's overall Nm is not accurate. However, Wright's estimate shows a very high level of gene flow within Pst. Incidentally, this calculation was also done including the sympatric Pst. The resulting estimate of Nm is much lower; only 3.32. This shows that the sympatric Pst samples were somewhat distant from other Pst. Gene flow into Wauconda only partly explains this. Closer examination of the F_{ST} calculations (Table 5.8) shows that sympatric Pst differed from allopatric Pst at the *Est-3*, *Odh*, *Pgi*, and *Pgm* loci. Since a shift in allele frequency at Wauconda was only found in *Pgi*, more than just the gene flow at Wauconda made the sympatric Pst genetically distant. The other sympatric Pst did not show signs of gene flow, yet they were somewhat

aberrant. Pairwise Slatkin's Nm estimates between all the populations of Pst (Table 5.10), also illustrate this. Pecten, Lynch and Wauconda Pst populations were all somewhat distant from the other Pst, thus lowering the overall Nm.

Summary

Nm estimates based on Wright's and Slatkin's techniques were different by almost an order of magnitude, but they exhibited similar trends. These values indicate that gene flow between allopatric Pfs-i and Pst is very low. There is no conclusive evidence that it is any higher in sympatry, at Pincher Creek. Although putative hybrids have been found in this area (see Chapter 4), they must be either infertile, strongly selected against, or so rare that gene flow is insignificant .

Gene flow levels between allopatric Pfs-c and Pst were slightly higher than for Pfs-i and Pst, and there was evidence that it was higher still at Wauconda. This was not entirely due to the hybrid specimen. Other Pfs-c and Pst there exhibited shifts of allele frequencies which made them more similar to each other.

The two morphs of Pfs were closer to each other than either was to Pst. Gene flow levels within Pst suggest that sympatric samples were slightly differentiated from other Pst.

5.3.5 Summary of Results

In allopatry, Pfs-i and Pst are morphologically and genetically distinct. In the Pincher Creek sympatric area, discriminant analysis and symmetry data suggested that gene flow may be taking place, but this was not conclusive. Genetic identities suggested that gene flow may be occurring there into Pst, but not into Pfs-i. Gene flow estimates indicated that introgression is no more extensive at Pincher Creek than in allopatry. Overall, there is no conclusive evidence of gene flow between Pfs-i and Pst at Pincher Creek, despite two putative hybrids being found there.

There was no evidence of gene flow at Cypress Hills.

There was stronger evidence of introgression between Pfs-c and Pst, at Wauconda. Discriminant analysis provided evidence of morphological introgression, and genetic identity data suggested that gene flow was occurring into Pst. Gene flow estimates also suggested that genetic introgression is occurring.

Nm estimates indicate that Pfs-i, Pfs-c, and Pst are distinct from each other, and that Pfs-c and Pfs-i are closer to each other than either is to Pst. Within Pst, sympatric populations from both Pincher Creek and Wauconda are somewhat distant from allopatric

populations. Other findings were that *Est-3* heterozygotes appear to be selected against, and that Chain Pst specimens are highly asymmetrical.

5.4 Discussion

How distinct are Pst, Pfs-i, and Pfs-c? In Chapter 3, differences between allopatric populations of these species and morphs were discussed. Their distinctiveness will be re-examined now in the light of sympatric site information, using estimates of Nm. Wright (1931) showed that, regardless of population size, one immigrant every two generations provides enough gene flow to prevent genetic drift. Thus if Nm is greater than 0.5, the populations will not diverge by genetic drift.

5.4.1 P. fossifrons versus P. stygicus

Levels of Gene Flow

Estimates of Nm indicate that gene flow between Pfs-i and Pst is occurring at such a low level that these taxa remain separate. There is more evidence that gene flow is occurring between Pfs-c and Pst, but according to Wright's criterion, it is not high enough to keep these taxa from diverging by genetic drift.

Selection Against Foreign Genes

There is evidence that foreign genes are being selected against in Pfs and Pst, which provides further evidence that they are separate species. Parental DNA which is not completely compatible results in offspring which are unfit. Because their mismatched DNA often results in deletions, inversions, and translocations, they often do not have two working copies of all genes. There is evidence that this is occurring in Pfs/Pst hybrids. Comparisons of heterozygosity to symmetry suggest that, in some sympatric populations, perhaps inbred specimens are developmentally more fit than genetically variable specimens. This indicates that some genetic information in the sympatric gene pool, perhaps foreign DNA, may be selected against.

The divergent nature of Pst populations from Wauconda and Lynch Lakes also provides evidence that there is some sort of selective pressure in sympatry, which does not exist in allopatry. In the Wauconda Pst, divergence was partly due to the inclusion of the hybrid specimen, but not entirely. It could just be coincidence that loss of (or failure to detect) rare alleles happens to have occurred in some sympatric populations rather than in allopatric ones. Or these samples could be slightly diverged due to unusual selection

pressures. This could be due to competition with Pfs, or due to the fact that these sites are at the edge of Pst range, where environmental conditions are marginal for their survival. However, it is also possible that pressure from introgressing foreign genes has caused this divergence.

The facts (discussed in Chapter 4) that no F_1 hybrids were found, and that the hybrid specimens which were found had deformed wings, provide further evidence that DNA from Pfs and Pst may not be completely compatible, resulting in selection against hybridization. The Wauconda hybrid specimen may also provide evidence of selection against foreign genes, depending upon which ancestry is correct. As discussed in Chapter 4, the "genetic error" theory of its ancestry involves the loss of genetic material. If this loss did occur, then surely there would be selection against the specimen and others like it.

Taxonomic Status

Does all this mean that Pfs and Pst are separate species? That depends which concept of species is accepted. The classical biological definition of species is based on reproductive isolation; any organisms which can produce fertile offspring are the same species (Mayr 1940). This has been developed into the phylogenetic concept of species (Nelson and Platnick 1981, p. 10) which defines any completely diagnosable group as a species. The existence of fertile Pfs/Pst hybrids indicates a grey area which does not conform to this definition of species. Recently, other hybridization studies have exposed similar grey areas between species, calling into question this rigid definition. As a result, the biological species concept has been criticized (Sokal and Crovello 1970), and other concepts of species have been developed (see Templeton 1989, for a review).

The recognition concept of species (Paterson 1978, 1985) limits a species to those individuals which are capable of reproduction, *and* which recognize each other as potential mates. A problem with this concept is that some organisms can form stable hybrid zones in areas of sympatry, yet still maintain their differences outside of those areas (for example, hybridization in swallowtails of the Papilio machaon species group; Sperling 1987).

A more recent idea is the cohesion concept of species (Templeton 1989), which states that a species is made up of organisms which have similar reproductive systems, and share the same fundamental niche. This allows for limited reproductive overlap, but one still must decide what range of variation to encompass within the limits of a species. There will always be individuals, and even whole populations, which live in slightly different environments, or have adapted to their environment slightly differently, so they do not share the same niche. Like previous concepts, the cohesion concept of species runs into difficulties because it attempts to restrict variable, changing entities to a single category.

The biological, recognition, and cohesion species concepts show a trend towards a more specific definition, but they still attempt to restrict all organisms into discrete positions. The evolutionary concept of species (Simpson 1961) defines a species as a group which shares a common evolutionary fate. This recognizes the dynamic nature of life, but the "evolutionary fate" of living organisms lies in the future, so it cannot be applied and tested in the present.

We need some notion of species, because we need to delimit groups of organisms if we want to study them. However, we have to keep in mind that these groups are artificial constructs. Sexual organisms are under two evolutionary pressures; the pressure to adapt to their environment and thus survive and reproduce, and the pressure to remain similar enough to other organisms that they can exchange genetic material. These opposing pressures are dynamic over time and space, and result in a patchy distribution of living things in multidimensional space, with a few individuals scattered between patches. We think of those patches as species, but no matter what parameters we use to categorize them, there will always be cases where a few individuals fall in between patches. A classification system with discrete compartments cannot always be applied to complex, living organisms. Thus we must accept a very unscientific notion; a concept which cannot be precisely defined. If we see species as collections of organisms which are similar, but not exactly the same, and that not all organisms fit nicely into these categories, we will be closer to reality.

From this viewpoint, I consider Pfs and Pst to be separate species. Individual specimens can usually be assigned to one or the other taxon, when several characters are examined. Hybrids are rare, and are strongly selected against, to preserve the separate gene pools. Because there are very few hybrids, this is actually a fairly clear-cut case; a more problematic situation may exist between the morphs of Pfs.

5.4.2 Status of P. fossifrons morphs

Wright's estimate of gene flow for Pfs-i versus Pfs-c shows that gene flow is not high enough between these morphs to prevent genetic drift. Although slight morphological and electrophoretic differences have been found, the division between Pfs-c and Pfs-i is not clearly defined. Reproductive isolation between the two morphs has not been demonstrated, so they will not be formally recognized taxonomically. It is likely that Pfs-c and Pfs-i can interbreed, but they could well accumulate enough differences in the future to become incapable if interbreeding. Though Pfs-c and Pfs-i are currently similar enough to be classed as the same species, they could well be species in the making; diverging at this very moment. These morphs illustrate that, within a group considered to be a species,

different populations can be responding to different environmental pressures, and can thus be evolving in different directions.

5.4.3 Homogeneity of P. stygicus

Wright's criterion shows that there is more than enough gene flow between populations of Pst for any to become reproductively isolated by genetic drift. Pst is clearly a much more homogeneous species than Pfs.

5.4.4 Other Questions

Selection against Est-3 *Heterozygotes*

Why are *Est-3* heterozygotes selected against, as indicated by their decreased symmetry in several populations? If selection against *Est-3* heterozygotes were strong enough, the Hardy-Weinberg equilibrium of these populations ought to have been affected. However, *Est-3* was not responsible for any more disequilibrium than other loci were (Table 2.2). The average probability of a sample's conformity to Hardy-Weinberg equilibrium at the *Est-3* locus was 0.584, compared to 0.334 for *Est-1* and 0.567 for *Pgm* (the other loci had probabilities greater than that for *Est-3*). Therefore, *Est-3* was not the most likely locus to be in disequilibrium. However, it is interesting to note that the one case of disequilibrium due to the *Est-3* locus was caused by a deficiency of heterozygotes. Although *Est-3* heterozygotes develop more asymmetrically, selection against them does not generally appear to be strong enough to cause significant mortality. These heterozygous beetles usually live; they just do not develop as smoothly.

Krisch (1971) claims that esterases have been implicated in detoxification reactions. It could be that different electromorphs interact in such a way that they are less efficient at detoxification of substances they encounter. Selection against heterozygotes has been found in other insects, for example the bark beetle Dendroctonus ponderosae, at the *Me* locus (Langor and Spence 1991).

Asymmetry of Chain Ponds Pst

Another question raised is, why were Chain Pst so asymmetrical? Except for the selection against *Est-3* heterozygotes, which was also found in other populations, there was no trend for heterozygotes in this population to be any more or less symmetrical than homozygotes. The population as a whole just has a lower level of symmetry than other populations of both Pst and Pfs. I think this has something to do with the habitat. This site

is at an altitude of approximately 1500m, and is heavily shaded by the Livingstone Mountains. Development of beetles there is at least two weeks behind that of other sites in southwestern Alberta. Perhaps the short summer exerts pressure on these specimens, and results in asymmetrical growth. This pressure must be stronger than the pressures associated with sympatry at Pincher Creek, because Chain Pst were less symmetrical than Pincher Creek sympatric populations.

Comparison of Nm Estimation Techniques

This chapter also illustrates the different strengths of Wright's and Slatkin's Nm estimating procedures. When several populations are being compared simultaneously, Wright's estimate can detect subgrouping of these populations, but Slatkin's cannot. However, Wright's estimate cannot detect which populations are subgrouped. Although overall Slatkin's estimates fail in this instance, pairwise Slatkin's estimates can detect the nature of these subgroupings.

Phylogenetic Positions of Pfs and Pst

A final series of questions raised by data examined in this chapter are; how are Pfs and Pst related phylogenetically? Are they sister species? Could Pst, Pfs-i, and Pfs-c be different surviving populations of an ancestral species, and thus form a monophyletic group, or could they have converged from different parts of the Patrobus phylogenetic tree? These questions cannot be answered by the results at hand, but Chapter 6 explores them further.

5.5 Summary

Morphological and electrophoretic data, including heterozygosity and symmetry information, were examined for indirect evidence of gene flow between Pfs and Pst. Estimates of gene flow were also calculated from electrophoretic data, using the techniques of Nei (1978), Wright (1951), and Slatkin (1985a).

Results were inconclusive for gene flow between Pfs-i and Pst. There is no clear evidence that gene flow is any greater in sympatry than in allopatry, despite the fact that two putative hybrids were found. There was more evidence of gene flow between Pfs-c and Pst. A hybrid specimen was found which was at least an F_2, indicating that at least some hybrids are fertile.

Gene flow between these taxa is at a low enough level that they remain distinct. Individuals of these species encounter one another at the sympatric sites, and occasionally

hybridize, but foreign genes are selected against in both Pfs and Pst. Evidence of selection against foreign genes includes the increased asymmetry of some sympatric populations of Pfs and Pst, the divergence of sympatric populations of Pst, the absence of the F_1 generation of hybrids, the reduced wings of hybrids, and one of the possible ancestries of the Wauconda hybrid specimen. Even though Pfs and Pst do not conform completely to the reproductive isolation concept of species, they remain classified as separate species, because strong barriers exist between them. They are an example of the grey areas which often exist between species, because our rigid species definitions do not always portray the complex, dynamic living world accurately.

An more uncertain situation was found within Pfs. Pfs-c and Pfs-i are fairly different, and gene flow is insufficient to keep them from diverging. These morphs are clearly moving in separate directions evolutionarily. However, no clear division could be found between them, so they are considered to be the same species. They are probably species in the making, and illustrate that not all individuals of a "species" are the same. These morphs have morphological, electrophoretic, and ecological differences, and because they are responding to different evolutionary pressures, they are on different evolutionary paths.

Populations of Pst were found to be homogeneous.

Other findings are that *Est-3* heterozygotes are less fit developmentally, and that Chain Pst specimens are particularly unfit developmentally. The former is hypothesized to be due to incompatibility of some *Est-3* alleles. The latter is hypothesized to be due to extreme environmental conditions at Chain Ponds.

Table 5.1: Test of a sample of 16 Rock Pond 'inland' P. fossifrons males for significance of bilateral variances. R1.1 = length of fused costa, subcosta, and radius; R1.2 = length of R_1 vein extending from R1.1; W1 = length of the cubital vein between the two cubito-anal cross-veins; W2 = width of the wedge cell. F = F-test result; df = degrees of freedom; p = probability that the measurement error variance is greater than the bilateral variance.

CHARACTER	MEASUREMENT ERROR (%)	MEASUREMENT ERROR VARIANCE	BILATERAL VARIANCE	F (df)	p
R1.1	0.8	0.62	10.348	16.696 (15,96)	0.0001
R1.2	5.7	1.062	2.525	2.376 (15,96)	0.0059
W1	0.5	0.349	4.515	12.937 (15,96)	0.0001
W2	2.3	0.188	1.82	9.705 (14,93)	0.0001

Table 5.2: Nei's (1978) unbiased pairwise genetic identities (above diagonal) and distances (below diagonal), between sympatric and pooled allopatric populations of Patrobus species and morphs. Values involving Wauconda populations are presented both without the hybrid specimen, and with the hybrid specimen divided between Pfs-c and Pst samples. See text for details of these calculations. Single dashes indicate comparisons which were not made.

POPULATION	1	2	3	4	5	6	7	8	9	10	11	12
'inland' P. fossifrons:												
1. pooled allopatric	---	1.0	1.0	1.0	.606	.603	.408	.408	.417	.423	.423	.429
2. Tern Pond	0	---	1.0	1.0	.594	.591	.400	.401	.410	.416	.416	.421
3. Rock Pond	0	0	---	1.0	.620	.617	.408	.408	.417	.424	.424	.431
4. Lynch Lakes	0	0	0	---	.599	.596	.407	.408	.416	.423	.423	.429
'coastal' P. fossifrons:												
5. Wauconda (no hybrid)	.500	.521	.477	.512	---	-	.497	.498	.486	.506	.493	-
6. Wauconda (incl. hybrid)	.506	.527	.483	.518	-	---	.501	.502	.489	.510	-	.524
P. stygicus:												
7. pooled allopatric	.898	.916	.897	.898	.700	.693	---	1.0	.999	.999	.978	.977
8. Lost Rd. Pond	.897	.914	.896	.897	.698	.691	0	---	.998	.999	.978	.976
9. Pecten Pond	.875	.893	.875	.877	.722	.716	.001	.002	---	.996	.968	.966
10. Lynch Lakes	.860	.877	.859	.860	.680	.673	.001	.001	.004	---	.968	.967
11. Wauconda (no hybrid)	.861	.878	.859	.861	.706	-	.023	.022	.032	.033	---	-
12. Wauconda (incl. hybrid)	.846	.865	.844	.848	-	.647	.025	.024	.035	.034	-	---

Table 5.3: Heterozygosities of Patrobus population samples. Wauconda heterozygosities are listed both without the hybrid, and with the hybrid split between 'coastal' P. fossifrons and P. stygicus. N = sample size; t = t-test for differences in heterozygosity between sympatric and pooled allopatric samples; d.f. = degrees of freedom; p = probability that the heterozygosities are similar.

Species & Morph: Population (N)	Heterozygosity ± SE	t-test vs. pooled allopatric samples t	d.f.	p
'inland' P. fossifrons				
pooled allopatric (6)	0.188±.028	-	-	-
Tern (14)	0.089±.028	2.501	14.3	0.025
Rock (40)	0.119±.019	2.018	10.7	0.071
Lynch (32)	0.137±.021	1.446	11.8	0.176
'coastal' P. fossifrons				
pooled allopatric (3)	0.250±.072	-	-	-
Wauconda (no hybrid) (18)	0.188±.027	0.810	2.6	0.503
Wauconda (+hybrid) (19)	0.191±.026	0.722	2.5	0.521
P. stygicus				
Chain (55)	0.109±.012	-	-	-
George (44)	0.088±.017	-	-	-
Hinton (23)	0.158±.021	-	-	-
pooled allopatric (127)	0.109±.009	-	-	-
Lost Rd. (53)	0.090±.014	1.175	97.3	0.243
Lynch (9)	0.111±.049	0.038	8.6	0.971
Pecten (8)	0.109±.037	0.003	7.9	0.998
Wauconda (no hybrid) (8)	0.141±.044	0.701	7.6	0.506
Wauconda (+hybrid) (9)	0.153±.040	1.049	8.8	0.325

Table 5.4: Bilateral variances (symmetries) of wing measurements, for large samples of Patrobus. Pfs-i = 'inland' P. fossifrons; Pst = P. stygicus. R1.1 = length of the sclerotized area of the fused costa, subcosta, and radius; R1.2 = length of the R_1 vein extending from R1.1; W1 = length of the cubital vein between the two cubito-anal cross veins; W2 = width of the wedge cell. Sample sizes are in brackets.

	CHARACTER			
POPULATION:	R1.1	R1.2	W1	W2
Lynch (Pfs-i)	1.143 (35)	1.088 (34)	2.714 (35)	0.426 (34)
Rock (Pfs-i)	2.344 (32)	1.067 (30)	2.152 (33)	0.276 (29)
Chain (Pst)	7.634 (41)	2.100 (40)	3.837 (43)	0.372 (41)
George (Pst)	3.385 (26)	1.917 (24)	1.594 (32)	0.328 (29)
Lost Rd. (Pst)	4.630 (46)	1.744 (43)	1.449 (49)	0.261 (45)

Table 5.5: F-tests of bilateral variances of wing measurements (Table 5.4) of large samples of Patrobus. Pfs-i = 'inland' P. fossifrons; Pst = P. stygicus. R1.1 = length of the sclerotized area of the fused costa, subcosta, and radius; R1.2 = length of the R_1 vein extending from R1.1; W1 = length of the cubital vein between the two cubito-anal cross veins; W2 = width of the wedge cell. Numbers in the table are F test results, with degrees of freedom in brackets. Direction of significant differences at a 95% confidence interval are indicated, with 'ns' indicating no significant difference.

	CHARACTER			
COMPARISON	R1.1	R1.2	W1	W2
Lynch (Pfs-i) vs.	2.051 (31,34)	1.020 (33,29)	1.261 (34,32)	1.543 (33,28)
Rock (Pfs-i)	Rock>Lynch	ns	ns	ns
Lynch (Pfs-i) vs.	6.679 (40,34)	1.930 (39,33)	1.414 (42,34)	1.145 (33,40)
Chain (Pst)	Chain>Lynch	ns	ns	ns
Lynch (Pfs-i) vs.	2.962 (25,34)	1.762 (23,33)	1.703 (34,31)	1.299 (33,28)
George (Pst)	George>Lynch	ns	ns	ns
Lynch (Pfs-i) vs.	4.051 (45,34)	1.603 (42,33)	1.873 (34,48)	1.632 (33,44)
Lost Rd. (Pst)	Lost Rd.>Lynch	ns	Lynch>Lost Rd.	ns
Rock (Pfs-i) vs.	3.257 (40,31)	1.968 (39,29)	1.783 (42,32)	1.348 (44,28)
Chain (Pst)	Chain>Rock	ns	ns	ns
Rock (Pfs-i) vs.	1.444 (25,31)	1.797 (23,29)	1.350 (32,31)	1.188 (28,28)
George (Pst)	ns	ns	ns	ns
Rock (Pfs-i) vs.	1.975 (45,31)	1.634 (42,29)	1.485 (32,48)	1.057 (28,44)
Lost Rd. (Pst)	ns	ns	ns	ns
Chain (Pst) vs.	2.255 (40,25)	1.095 (39,23)	2.407 (42,31)	1.134 (40,28)
George (Pst)	Chain>George	ns	Chain>George	ns
Chain (Pst) vs.	1.649 (40,45)	1.204 (39,42)	2.648 (42,48)	1.425 (40,44)
Lost Rd. (Pst)	ns	ns	Chain>Lost Rd.	ns
George (Pst) vs.	1.368 (45,25)	1.099 (23,42)	1.100 (31,48)	1.257 (28,44)
Lost Rd. (Pst)	ns	ns	ns	ns

Table 5.6: Bilateral variances of homozygotes (hom) and heterozygotes (het) at various loci, for large samples of Patrobus. R1.1 = length of the sclerotized area of the fused costa, subcosta, and radius; R1.2 = length of the R_1 vein extending from R1.1; W1 = length of the cubital vein between the two cubito-anal cross veins; W2 = width of the wedge cell. Comparisons with less than three homozygotes and heterozygotes are deleted. F-test results indicate direction of significant differences at a 95% confidence interval, with 'ns' signifying no significant difference.

Population		CHARACTER			
Locus	Group	R1.1	R1.2	W1	W2
Lynch ('inland' P. fossifrons):					
Est-1:	homozygotes	0.941 (17)	2.588 (17)	3.833 (18)	0.338 (17)
	heterozygotes	1.267 (15)	1.500 (14)	1.600 (15)	0.589 (14)
	F-test	ns	ns	ns	ns
Est-3:	homozygotes	1.238 (21)	1.650 (20)	1.429 (21)	0.263 (19)
	heterozygotes	1.000 (14)	2.714 (14)	4.643 (14)	0.633 (15)
	F-test	ns	ns	het > hom	ns
Odh:	homozygotes	1.100 (30)	2.200 (30)	3.033 (30)	0.425 (30)
	heterozygotes	1.400 (5)	1.250 (4)	0.800 (5)	0.438 (4)
	F-test	ns	ns	ns	ns
Pgm:	homozygotes	1.094 (32)	2.129 (31)		0.379 (31)
	heterozygotes	1.667 (3)	1.667 (3)	N/A	0.917 (3)
	F-test	ns	ns		ns
Rock ('inland' P. fossifrons):					
Est-1:	homozygotes	2.154 (26)	1.375 (24)	2.080 (25)	0.202 (21)
	heterozygotes	3.167 (6)	5.833 (6)	2.375 (8)	0.469 (8)
	F-test	ns	het > hom	ns	ns
Est-3:	homozygotes	1.611 (18)	0.938 (16)	2.111 (18)	0.339 (14)
	heterozygotes	3.286 (14)	3.786 (14)	2.200 (15)	0.217 (15)
	F-test	ns	het > hom	ns	ns
Odh:	homozygotes	2.080 (25)	1.542 (24)	1.692 (26)	0.239 (23)
	heterozygotes	3.286 (7)	5.167 (6)	3.857 (7)	0.417 (6)
	F-test	ns	het > hom	ns	ns
Chain (P. stygicus):					
Est-1:	homozygotes	6.621 (29)	7.931 (29)	4.600 (30)	0.343 (27)
	heterozygotes	10.08 (12)	4.182 (11)	2.077 (13)	0.429 (14)
	F-test	ns	ns	ns	ns
Est-3:	homozygotes	6.968 (31)	4.300 (30)	1.848 (33)	0.383 (32)
	heterozygotes	9.700 (10)	14.70 (10)	10.40 (10)	0.333 (9)
	F-test	ns	het > hom	het > hom	ns
Odh:	homozygotes	7.289 (38)	7.000 (37)	4.075 (40)	0.368 (38)
	heterozygotes	12.00 (3)	5.667 (3)	0.667 (3)	0.417 (3)
	F-test	ns	ns	ns	ns
Pgm:	homozygotes	7.472 (36)			0.399 (37)
	heterozygotes	9.000 (3)	N/A	N/A	0.167 (3)
	F-test	ns			ns

Table 5.6, continued.

George (P. stygicus):					
Est-1:	homozygotes	3.714 (21)	6.238 (21)	1.577 (26)	0.326 (23)
	heterozygotes	2.000 (5)	5.667 (3)	1.667 (6)	0.125 (6)
	F-test	ns	ns	ns	ns
Est-3:	homozygotes	2.227 (22)	4.250 (20)	1.750 (28)	0.330 (25)
	heterozygotes	9.750 (4)	15.75 (4)	0.500 (4)	0.313 (4)
	F-test	het > hom	ns	ns	ns
Pgm:	homozygotes	3.727 (22)	6.250 (20)	1.654 (26)	0.260 (24)
	heterozygotes	1.667 (3)	6.333 (3)	1.400 (5)	0.813 (4)
	F-test	ns	ns	ns	ns
Lost Rd. (P. stygicus):					
Est-1:	homozygotes	3.758 (33)	5.097 (31)	1.583 (36)	0.250 (32)
	heterozygotes	6.846 (13)	5.250 (12)	1.077 (13)	0.288 (13)
	F-test	ns	ns	ns	ns
Est-3:	homozygotes	3.514 (37)	4.265 (34)	1.667 (39)	0.201 (36)
	heterozygotes	9.222 (9)	8.444 (9)	0.600 (10)	0.500 (9)
	F-test	het > hom	ns	ns	ns
Pgm:	homozygotes	4.643 (42)	4.769 (39)	1.511 (45)	0.274 (42)
	heterozygotes	4.500 (4)	8.750 (4)	0.750 (4)	0.083 (3)
	F-test	ns	ns	ns	ns

Table 5.7: Estimates of gene flow between Patrobus species and morphs. D = Nei's (1978) unbiased genetic distance; m = proportion of immigrants per generation; F_{ST} = Wright's (1951) correlation coefficient; Nm = number of immigrants per generation; $\bar{P}_1$ = average frequency of private alleles (Slatkin; 1985a).

	Nei		Wright		Slatkin	
COMPARISON:	D	m	F_{ST}	Nm	$\bar{P}_1$	Nm
'coastal' P. fossifrons vs. P. stygicus						
allopatric	0.828	1.55×10^{-6}	0.490	0.260	0.348	0.012
Wauconda (no hybrid)	0.706	1.95×10^{-6}	0.516	0.234	0.575	0.042
Wauconda (+ hybrid)	0.647	2.20×10^{-6}	0.469	0.286	0.544	0.046
'inland' P. fossifrons vs. P. stygicus						
allopatric	0.898	1.37×10^{-6}	0.673	0.121	0.378	0.009
Lost Rd. & Pecten vs. Rock	0.886*	1.40×10^{-6}*	0.689	0.113	0.411	0.018
Lost Rd. & Pecten vs. Tern	0.904*	1.36×10^{-6}*	0.729	0.093	0.396	0.022
Lynch Lakes	0.860	1.47×10^{-6}	0.668	0.124	0.503	0.035
'coastal' vs. 'inland' P. fossifrons						
allopatric populations	0.319	5.32×10^{-6}	0.329	0.510	0.491	0.204
all populations	0.467	3.36×10^{-6}	0.414	0.354	0.144	0.650
intraspecific P. stygicus						
allopatric populations	--	--	0.014	17.6	0.025	4.98
all populations	--	--	0.070	3.32	0.006	61.2

* based on averages of pairwise comparisons.

Table 5.8: Wright's (1951) F-statistics of electrophoretic data, for species and morphs of Patrobus. F_{IS} = fixation index of individuals relative to subpopulations; F_{IT} = fixation index of individuals relative to the total population; F_{ST} = fixation index of subpopulations relative to the total population.

'coastal' P. fossifrons vs. P. stygicus:	allopatric populations			Wauconda (including hybrid)		
LOCUS	F_{IS}	F_{IT}	F_{ST}	F_{IS}	F_{IT}	F_{ST}
Ao	–	1.000	1.000	-0.040	0.924	0.927
Apk	0.977	0.981	0.194	–	–	–
Est-1	0.646	0.805	0.450	0.593	0.797	0.501
Est-3	0.360	0.617	0.401	0.097	0.378	0.312
Hk	-0.025	-0.012	0.012	–	–	–
Odh	-0.444	0.419	0.598	0.582	0.821	0.572
Pgi	0.290	0.483	0.271	-0.219	-0.153	0.054
Pgm	-0.359	0.166	0.386	0.091	0.326	0.260
Mean	0.254	0.619	0.490	0.221	0.588	0.469
'inland' P. fossifrons vs. P. stygicus:	allopatric populations			Lynch Lakes		
LOCUS	F_{IS}	F_{IT}	F_{ST}	F_{IS}	F_{IT}	F_{ST}
Ao	–	1.000	1.000	–	1.000	1.000
Apk	-0.005	-0.003	0.003	–	–	–
Est-1	-0.114	0.428	0.487	0.040	0.441	0.418
Est-3	-0.309	0.352	0.505	0.080	0.590	0.554
Hk	-0.025	-0.012	0.012	-0.014	-0.007	0.007
Odh	-0.072	0.795	0.809	-0.160	0.608	0.662
Pgi	0.059	0.915	0.910	-0.059	0.894	0.900
Pgm	-0.026	-0.020	0.006	-0.043	-0.021	0.021
Mean	-0.149	0.624	0.673	0.002	0.668	0.668
intraspecific P. stygicus:	allopatric populations			all populations		
LOCUS	F_{IS}	F_{IT}	F_{ST}	F_{IS}	F_{IT}	F_{ST}
Ao	–	–	–	–	–	–
Apk	-0.017	-0.007	0.010	-0.015	-0.004	0.010
Est-1	0.125	0.140	0.017	0.172	0.195	0.027
Est-3	0.056	0.070	0.050	0.001	0.148	0.147
Hk	-0.035	-0.030	0.005	-0.033	-0.018	0.015
Odh	-0.039	-0.028	0.010	-0.112	-0.034	0.070
Pgi	0.046	0.061	0.015	-0.101	-0.028	0.066
Pgm	-0.016	-0.004	0.012	0.136	0.178	0.049
Mean	0.050	0.063	0.014	0.052	0.118	0.070

Table 5.9: Slatkin's (1985a) pairwise Nm estimates between Patrobus fossifrons populations. Pfs-c = 'coastal' P. fossifrons; Pfs-i = 'inland' P. fossifrons. $\#P_1$ = number of private alleles; $\bar{N}$ = average sample size; $\bar{P}_1$ = average frequency of private alleles; Nm = estimated number of individuals per generation which are migrants, corrected for a sample size of 25.

COMPARISON	$\#P_1$	$\bar{N}$	$\bar{P}_1$	Nm
allopatric Pfs-c vs. Wauconda (Pfs-c)	7	9.43	0.270	0.283
allopatric Pfs-i vs. Tern (Pfs-i)	3	10.33	0.073	3.428
allopatric Pfs-i vs. Rock (Pfs-i)	2	52.50	0.043	1.946
allopatric Pfs-i vs. Lynch (Pfs-i)	1	35.00	0.014	25.654
Lynch (Pfs-i) vs. Rock (Pfs-i)	3	46.67	0.033	3.600
Lynch (Pfs-i) vs. Tern (Pfs-i)	4	31.50	0.045	3.004
Rock (Pfs-i) vs. Tern (Pfs-i)	5	45.80	0.050	1.617
allopatric Pfs-c vs. allopatric Pfs-i	9	4.00	0.491	0.204
allopatric Pfs-c vs. Lynch (Pfs-i)	10	16.10	0.443	0.062
allopatric Pfs-c vs. Rock (Pfs-i)	7	23.57	0.381	0.057
allopatric Pfs-c vs. Tern (Pfs-i)	10	7.30	0.475	0.119
Wauconda (Pfs-c) vs. allopatric Pfs-i	8	13.50	0.587	0.042
Wauconda (Pfs-c) vs. Lynch (Pfs-i)	9	25.89	0.519	0.028
Wauconda (Pfs-c) vs. Rock (Pfs-i)	6	35.33	0.505	0.022
Wauconda (Pfs-c) vs. Tern (Pfs-i)	9	17.78	0.592	0.032

Table 5.10: Slatkin's (1985a) pairwise Nm estimates between Patrobus stygicus populations. $\#P_1$ = number of private alleles; $\bar{N}$ = average sample size; $\bar{P}_1$ = average frequency of private alleles; Nm = estimated number of individuals per generation which are migrants, corrected for a sample size of 25.

COMPARISON	$\#P_1$	$\bar{N}$	$\bar{P}_1$	Nm
Chain vs. George	2	81.00	0.006	58.111
Chain vs. Hinton	3	53.67	0.014	17.175
Chain vs. Long	2	52.50	0.033	3.334
Chain vs. Lost Rd.	3	67.00	0.020	7.131
Chain vs. Lynch	8	60.13	0.038	2.098
Chain vs. Pecten	7	68.14	0.037	1.980
Chain vs. Wauconda	7	68.14	0.034	2.352
George vs. Hinton	3	78.33	0.016	9.051
George vs. Long	4	70.25	0.017	8.989
George vs. Lost Rd.	3	78.33	0.014	12.682
George vs. Lynch	10	67.50	0.032	2.737
George vs. Pecten	9	73.11	0.019	6.960
George vs. Wauconda	9	73.11	0.025	4.198
Hinton vs. Long	5	32.20	0.022	11.831
Hinton vs. Lost Rd.	4	54.75	0.024	6.109
Hinton vs. Lynch	7	22.14	0.057	2.643
Hinton vs. Pecten	6	24.67	0.054	2.569
Hinton vs. Wauconda	8	22.50	0.048	3.584
Long vs. Lost Rd.	3	54.67	0.010	33.086
Long vs. Lynch	6	32.50	0.029	6.972
Long vs. Pecten	9	29.22	0.040	3.943
Long vs. Wauconda	7	31.43	0.044	3.049
Lost Rd. vs. Lynch	7	64.57	0.013	16.086
Lost Rd. vs. Pecten	8	64.75	0.017	9.435
Lost Rd. vs. Wauconda	8	64.75	0.028	3.676
Lynch vs. Pecten	5	8.60	0.129	1.334
Lynch vs. Wauconda	3	9.00	0.130	1.266
Pecten vs. Wauconda	6	8.50	0.148	1.029

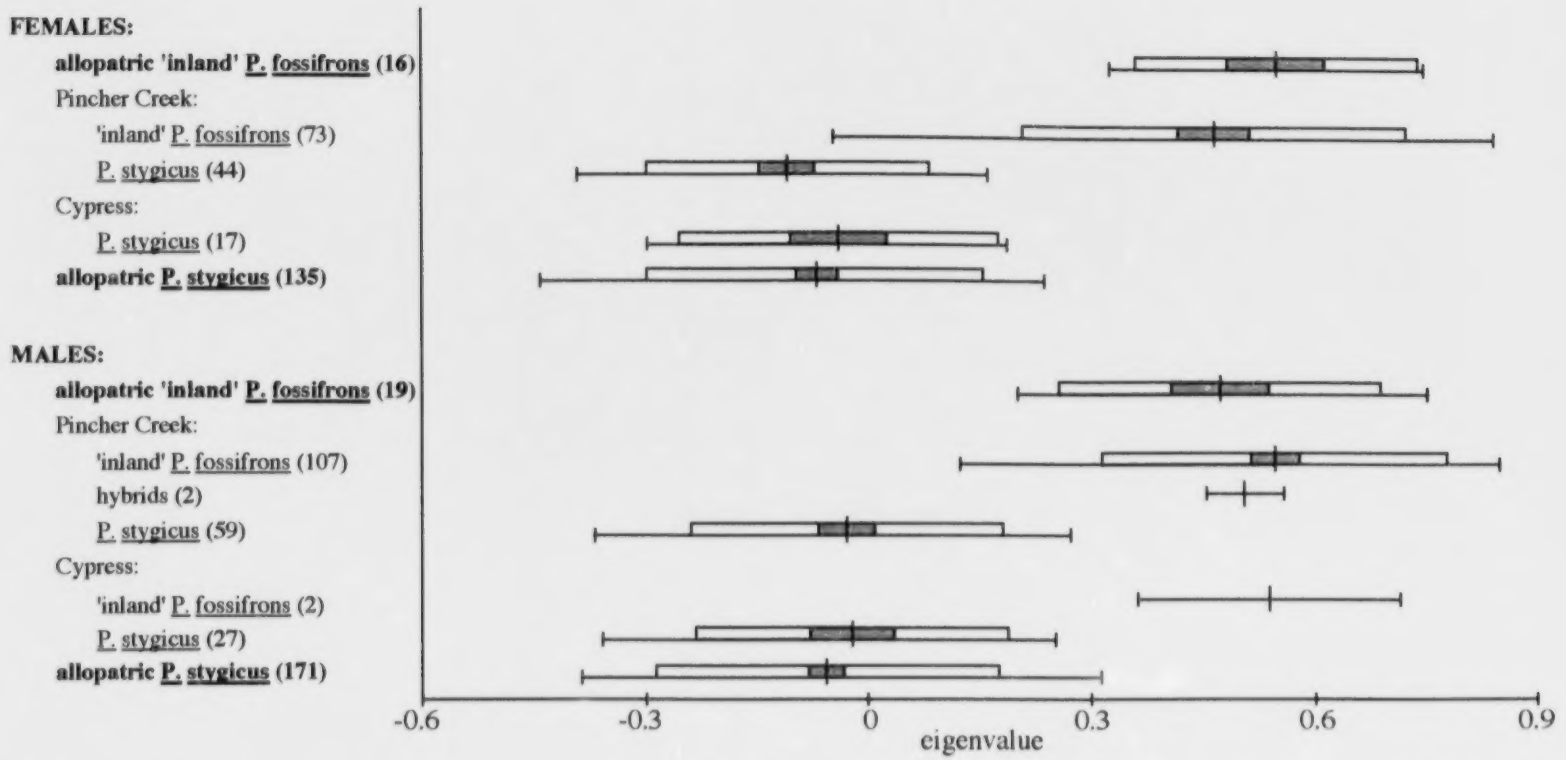


Figure 5.1: Multivariate comparison of Patrobus stygicus and 'inland' P. fossifrons. Sample sizes are in brackets. Eigenvalues are based on a discriminant analysis of four morphological measurements. Figure based on the procedure of Hubbs and Hubbs (1953)*.

* Horizontal line = range of measurements; vertical line = mean; outlined box = mean ± 1.5 standard deviations; shaded box = mean ± 2 standard errors.

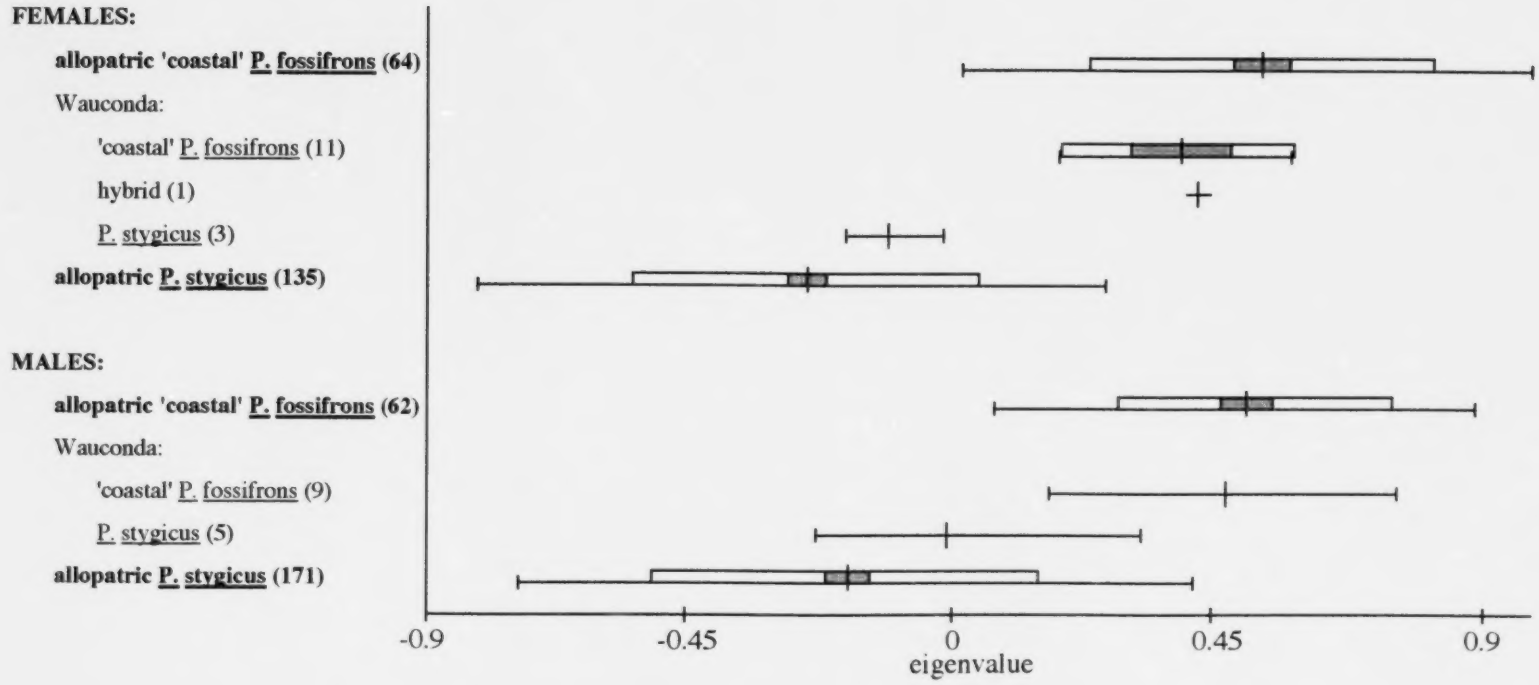


Figure 5.2: Multivariate comparison of Patrobus stygicus and 'coastal' P. fossifrons. Sample sizes are in brackets. Eigenvalues are based on a discriminant analysis of four morphological measurements. Figure based on the procedure of Hubbs and Hubbs (1953)*.

* Horizontal line = range of measurements; vertical line = mean; outlined box = mean ± 1.5 standard deviations; shaded box = mean ± 2 standard errors.

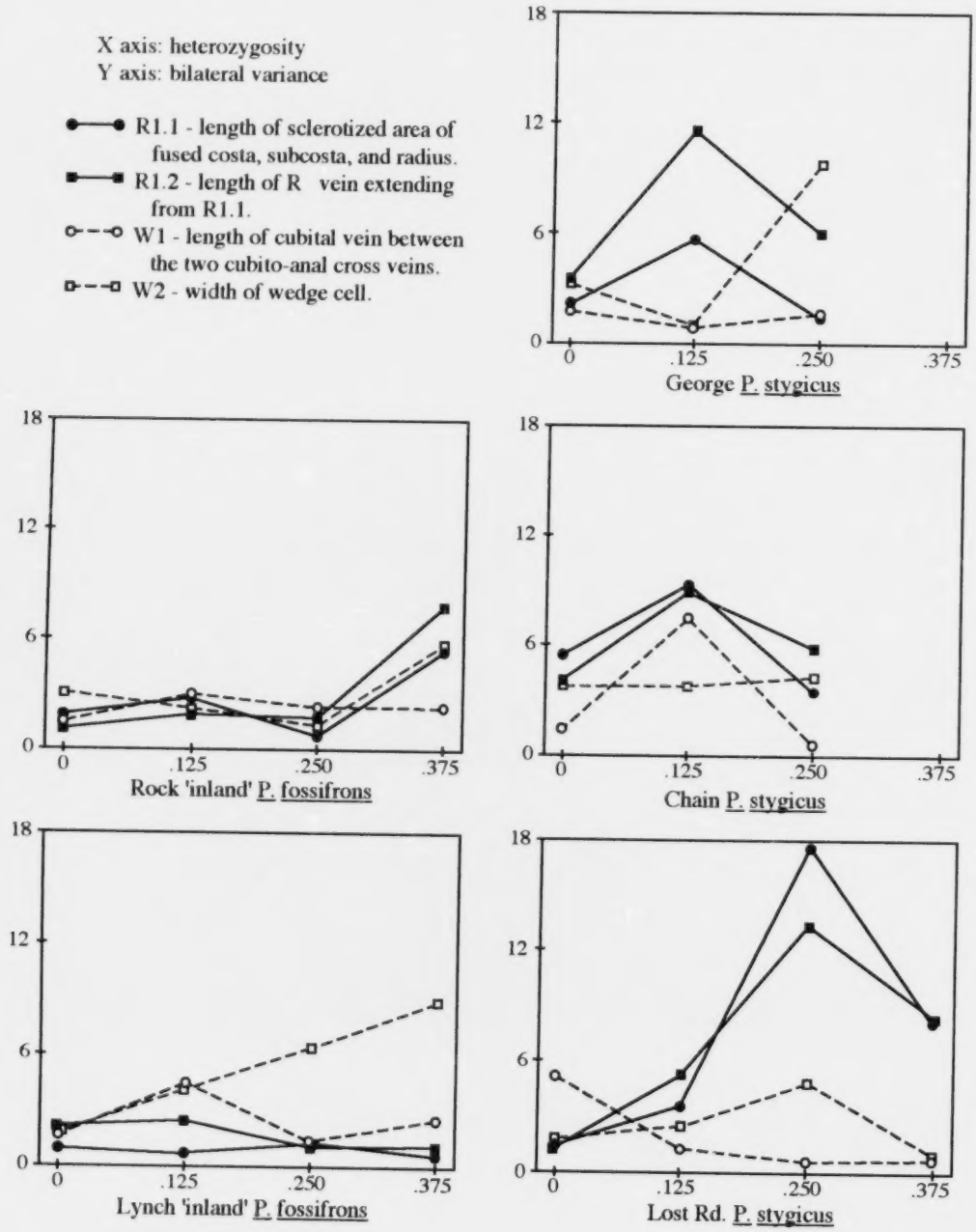


Figure 5.3: Heterozygosity versus bilateral variance of wing measurements, in Patrobus populations. Data for character W2 are10X the bilateral variance. Measurement error variances are 0.62, 1.062, 0.349, and 0.188, respectively, for R1.1, R1.2, W1, and W2.

6. RELATIONSHIPS IN THE GENUS PATROBUS

6.1 Introduction

During the course of this project, specimens of all six North American species of Patrobus were collected, as well as of the European P. atrorufus. Since electrophoretic data were being collected from these specimens, an opportunity was at hand to test the currently-accepted phylogeny of Patrobus, based on morphological characters.

The current understanding of relationships among the Patrobus species examined here (Figure 1.2) is based primarily on the work of Darlington (1938), Kühnelt (1941), and Lindroth (1961). (For a discussion of these studies, see Chapter 1.) These workers relied primarily on examination of the male genitalia to formulate their hypotheses. Despite the fact that this structure provides many useful characters, it is limited to a single system. To be reliable, a phylogenetic data set ought to employ characters from as many different functional systems as possible. From this perspective, the electrophoretic data set examined here is also very limited, but it provides an independent test of the morphologically-based hypothesis.

6.2 Theory

6.2.1 Morphological versus Electrophoretic Characters

Both morphological and electrophoretic characters are susceptible to homoplasies, due to random effects as well as to selection of certain morphological or molecular configurations. Neither form of data is inherently superior to the other; the value of each depends entirely on the sort of problems being explored.

In morphological studies, characters can generally be described and ordered into transformation series for phylogenetic analysis. Electrophoretic characters are more difficult to treat. Electrophoretic data consist of different alleles or electromorphs in different combinations at various loci. Currently there is much debate about which of these is the 'character', what constitutes the 'character state', and how the character states ought to be organized into a transformation network.

6.2.2 Genetic Distance Analysis

One solution to the problems posed above is to do a phenetic analysis based on pairwise distance measures. The most widely used distance measures are Nei's (1972, 1978) distances, Rogers' (1972) distance, Cavalli-Sforza and Edwards (1967) arc and chord distances, and the Prevosti, or Manhattan (Wright 1978) distance.

Nei's and Rogers' distances measure differences in homozygosity, while the others measure differences in heterozygosity. Nei's and Rogers' distances were criticized by Wright (1978) and Hillis (1984) for being unduly influenced by the levels of heterozygosity within populations. As well, Nei's distances are nonmetric, so they are not indicative of the actual amount of evolutionary change which has been observed (Farris 1981). Nei's, Rogers', and Prevosti's distances also have a problem in that they consider equal differences in allele frequency as equally important. This is undesirable, because it causes these measures to underestimate the importance of unique alleles (Wright 1978, Swofford and Olsen 1990). Overall, I feel that the Cavalli-Sforza and Edwards arc distance is superior. It is expressed as:

[6.1]
$$D = \sqrt{(1/L)\bullet\sum_{L}(2\cos^{-1}\bullet\sum_{i}\sqrt{X_i Y_i}/\pi)^2}$$

where **D** is the resulting distance measure, **L** is the number of loci, **i** is the number of alleles at locus L, and **X** and **Y** are the frequencies of alleles i in the two populations being compared.

Distance Phenogram Construction

After selecting a distance measure, one must choose a method of tree construction. One technique, cluster analysis, is the successive combining of the two most similar taxa or groups of taxa into one group, until all the taxa are connected. This method has been criticized by Farris (1981) and Swofford and Olsen (1990) for its assumption of a constant rate of evolution.

Additive methods such as that of Fitch and Margoliash (1967) correct for unequal rates of evolution. These methods search for the shortest tree by minimizing the percent standard deviation of the tree. However, Farris (1981) and Swofford and Olsen (1990) criticize both the Cluster and Additive techniques, for allowing negative branch lengths. As this cannot be possible in nature, these methods do not portray evolution accurately.

Both Farris (1981) and Swofford (1981) recommend the distance Wagner analysis, a maximum-likelihood estimate of phylogeny (Kluge and Farris 1969). It does not assume a set rate for evolution, and it does not allow branch lengths to be less than the actual observed distance between taxa. Just as phylogenetic analysis of discrete characters seeks to minimize homoplastic events, Wagnerian distance analysis minimizes branch lengths, with the observed genetic distances as minimum values. I feel that it is the best phenetic tree building method.

6.2.3 Discrete Character Analysis

Since phenetic techniques do not accurately portray evolutionary processes, many attempts have been made to apply phylogenetic techniques to electrophoretic analysis. However, two major problems arise: 1, whether to treat the locus or the allele as the character, and 2, whether to use allele frequency data or simple presence/absence as the character states.

Loci As Characters

Mickevich and Mitter (1981, 1982), and Buth (1984) contend that the locus is the unit undergoing evolution, so it should be the character. This recognizes that the alleles at a locus are not entirely independent, since one allele could replace other alleles at that locus, either by selection or random drift.

One form of analysis treating the locus as character, used by Richardson and Smouse (1976) and Richardson *et al.* (1977), is to treat the weighted average of all electromorph mobilities at a locus as a phenotype. Because this procedure makes the contentious assumption that there is phylogenetic information in the mobilities, and because fast and slow electromorphs can cancel each other out, and not have any influence on the average mobility, I do not feel that it is valid.

Another technique is to treat the various combinations of alleles, and possibly their frequencies, as the character states. Attempts have been made to order these character states into transformation series on the basis of relative mobility (Ohta and Kimura 1973), or by putting different weights on losses and gains of alleles (Mickevich and Mitter 1981, 1982). Another method (Mickevich and Mitter 1981, 1982) builds a transformation sequence and a phylogeny from the same data, and then uses each to support the other. This method has yet to be described adequately by the authors, and appears to be based on circular reasoning.

A fundamental problem with all of these techniques treating the locus as the character is that, while frequencies of alleles at a locus are somewhat dependent on each other, the presence or absence of alleles in a population is not. Alleles can appear or disappear independently of other alleles at that locus, resulting in complex relationships between allele combinations. This can result in what I call multidimensional transformation networks rather than linear transformation series. I feel that, to be useful in phylogenetic analysis, electrophoretic loci must be arranged as simpler characters which form one-dimensional transformation series.

Alleles As Characters

To analyze transformation networks, some workers have suggested that the alleles be considered as the characters. Frequencies, or simple 'presence/absence' of alleles can then be used as the character states.

Use of frequencies has been criticized because they are subject to major changes over very short periods of time (Crother 1990), and because it would cause the characters (the alleles), to be dependent on each other (Mickevich and Johnson 1976, Farris 1981). Mickevich and Johnson (1976) suggested that, to make alleles independent of one another, they be treated as binary characters, with the two states being 'present' and 'absent'. The resulting collection of independent characters at a locus would then be similar to the complex locus-character discussed previously, except that all the complex branches of the transformation network would be supported by individual character synapotypies. I feel that this is an accurate, systematic way to portray these transformation networks.

A problem with the 'alleles-as-characters' model is sampling error. Unless samples are very large, it is possible to code rare alleles as 'absent' when in fact they are present at low frequencies. The effect of not detecting rare alleles is that taxa represented by small sample sizes would be artificially distant in the analysis. To avoid overlooking rare alleles, Mickevich and Johnson (1976) suggested that alleles with a frequency of less than 0.05 be excluded from analysis. However, some synapotypies can be missed when the rare alleles are deleted. As well, ignoring all rare alleles implies that alleles with low frequencies are less important than those with higher frequencies. This goes against all the arguments that frequencies are not useful; how can frequencies tell us which alleles are important, if they are subject to great change within evolutionary time? The drawbacks of both of these techniques are a necessary evil with the current understanding of electrophoretic data.

Overall, I feel that the absolute criterion has more validity when samples are very large, because the chances of not detecting rare alleles in large samples is quite small. However,

with smaller samples (< 25 individuals), I feel that the 5% criterion is probably better than absolute 'presence/absence'.

Phylogeny Reconstruction

As in phenetic analysis, after determining characters and character states, discrete character data are used to construct the most parsimonious tree. The best analysis would be an exhaustive search of all possible tree topologies, for the one with the fewest homoplasies. Unfortunately, even today's powerful computers cannot handle exhaustive searches of even moderately sized (20 taxa or so) data sets, so phylogenies often have to be estimated by some other technique, such as Wagner analysis.

6.2.4 Summary

Electrophoretic and morphological data have different strengths and weaknesses, with neither being inherently superior. Analyzing morphological data is usually straightforward, but there are several approaches to electrophoretic data analysis, none of which is universally accepted.

After comparing several pairwise distance analysis techniques, I conclude that the most reliable method is to use the Cavalli-Sforza and Edwards arc distance in a distance Wagner analysis.

I feel that phylogenetic analysis of discrete characters is superior to any distance-based technique, because it more accurately reflects the process of evolution. Although the locus intuitively seems to be the character, treating it as such results in problems relating character states to one another. I feel that analysis is best done with the alleles as binary characters, coded as 'present' or 'absent'.

6.3 Materials and Methods

6.3.1 Specimens Examined

Electrophoretic data were collected from 'coastal' and 'inland' P. fossifrons, P. stygicus, P. lecontei, P. longicornis, P. foveocollis, P. septentrionis, and P. atrorufus. Unfortunately, specimens of the most closely-related genus to Patrobus, Platypatrobus, were not collected, so the next-closest genus Diplous was used as an outgroup. Two Diplous species were collected: D. aterrimus and D. californicus.

Specimens were scored for electromorphs at eight loci, and allele frequencies of the various samples were calculated. Samples were then tested for conformity to Hardy-Weinberg equilibrium. For the most part, specimens within samples, and in different days' samples from the same site were found to be randomly mating and not under any selective pressure, for the eight loci sampled. Refer to Chapter 2 for details on this data gathering and preliminary analysis. Allele frequencies at sampled sites appear in Tables 2.5 and 2.6.

6.3.2 Distance Analysis Procedure

As discussed above, Nei's (1972, 1978) genetic identity measures are considered here to be inferior to the Cavalli-Sforza and Edwards arc distance. However, many studies of other organisms are based on Nei's (1972) measure. Therefore, Nei's (1972) measures were calculated for comparison to other studies, and Cavalli-Sforza and Edwards arc distances were calculated for analysis via the distance Wagner technique.

Distances were calculated between all population samples with at least five specimens[1], using the FORTRAN computer program BIOSYS-1, version 1.7 (Swofford and Selander 1981). The distance Wagner analysis was done using the DISWAG subroutine of BIOSYS-1, with parameters set as follows:

ADDCRIT = 0. This uses the multiple addition criterion (Swofford 1981) for adding successive taxa to the tree. This means that taxa are added by all three methods allowed by the program, with all of the shortest trees being saved for further steps.

FITCRIT = 1. This uses Prager and Wilson's (1976) F value to determine which partial trees are best, for use in further steps of tree construction.

MAXTREE = 30. This is the maximum the program allows. It determines how many partial trees are to be saved between successive steps in tree construction.

This analysis yielded unrooted trees, which were then rooted at the middle of the branch connecting the outgroup to the rest of the taxa.

[1] Note that this excludes P. foveocollis from the phenetic analysis, since only two specimens were collected.

6.3.3 Discrete Character Analysis Procedure

For the discrete character analysis, each of the 75 alleles was treated as an independent binary character. Two separate analyses were made. In the first, alleles with frequencies of at least 0.05 were considered to be 'present', while those with frequencies of less than 0.05 were 'absent'. In the second analysis, absolute presence and absence was used. All population samples within species were pooled for these discrete analyses, except for the two morphs of P. fossifrons, which were kept separate. From these initial data sets, alleles present in all taxa, or in only one taxon, were removed, since they are phylogenetically uninformative, and serve only to increase computer running time. The Diplous species, pooled together into a single taxon, were used as the outgroup.

These data sets were analysed with the branch-and-bound algorithm PENNY, on Felsenstein's (1985) PHYLIP phylogeny inference package, version 3.1, on a Macintosh Plus® computer. This program finds the tree(s) with the fewest evolutionary steps, which is assumed to be the most parsimonious estimate of phylogeny. Assumptions are that it is just as evolutionarily costly to gain an allele as it is to lose it, and that all alleles are equally costly to gain or lose.

The PENNY algorithm results in unrooted trees, so they were rooted with Diplous as the most distantly related taxon in the phylogeny.

6.4 Results

6.4.1 Distance Analysis

Nei's genetic identities and Cavalli-Sforza and Edwards arc distances appear in Table 6.1.

The shortest tree resulting from the distance Wagner analysis of Cavalli-Sforza and Edwards arc distances is illustrated in Figure 6.1. Other trees which were almost as short had the same branching arrangement at the species level, and only slight rearrangements of populations within P. stygicus. Therefore, the tree pictured is clearly the best hypothesis with the data at hand.

The Cavalli-Sforza and Edwards arc distance tree was compared to trees generated using the Cavalli-Sforza and Edwards Chord, Rogers, Modified Rogers, Prevosti, and Edwards distances. These trees all had the same arrangement of species, except for some uncertainty as to the placement of P. atrorufus.

6.4.2 Discrete Character Analysis

Binary-coded data, using absolute presence/absence and the 5% criterion, appear in Table 6.2.

When the absolute presence/absence data were analysed, 13 equally parsimonious trees were produced. The species P. atrorufus, P. lecontei and P. longicornis appeared in various positions in these trees. The consensus tree appears in Figure 6.2. The analysis using the '5% criterion' to determine electromorph presence produced five equally parsimonious trees. Figure 6.3 is the consensus, where the placement of P. lecontei is the only major question.

6.5 Discussion

6.5.1 Age and Status of the Taxa

The taxonomic levels of classification currently used in the Patrobini were examined using Nei's (1972) genetic identity values (Table 6.1). Thorpe (1979, 1983) compiled measures from many studies of invertebrate populations, and reported that populations within the same species tended to have identities greater than 0.9, species within the same genus tended to have identities of 0.25 to 0.85, and species in different genera tended to have identities of less than 0.3. He also concluded that an identity of 0.0 corresponded to 18 to 20 million years of evolutionary divergence, given a constant rate of evolution. By this measure, the minimum age of a species would be about three million years.

The morphological similarity of Patrobus species gives the initial impression that they are not very old. However, genetic distance measures suggest the opposite. P. stygicus is sufficiently distinct from the two morphs of P. fossifrons to be ranked as a separate species, with mean genetic identities of approximately 0.4 to 0.6. P. lecontei and P. longicornis are distinct enough from some members of Patrobus that they could be placed in their own genus. As well, P. atrorufus and P. septentrionis are diverged enough that they could be in separate genera.

Within P. fossifrons, the 'coastal' and 'inland' morphs are distinct enough by Thorpe's criterion that they could be classified as ranked species. However, as discussed in Chapter 3, no clear division could be found between them, so they are considered to be the same species.

Thorpe's numbers are only rough guidelines for classification levels. They should not be adhered to rigidly, since evolution proceeds at different rates in different groups. I do

not propose changing the classification levels of any of these taxa. However, these numbers tell us that either Patrobus is a very old group, or that Patrobus species are diverging more rapidly than most insects. I postulate the former. The fossil record indicates that carabids are differentiating very slowly, compared to other insects (Kavanaugh 1979). Hopkins *et al.* (1971) found fossil evidence that many extant North American carabid species have existed essentially unchanged for up to 5.7 million years, and Matthews (1979a) found carabid fossils dating from approximately eight million years ago, which were very similar to extant carabid species.

By all indications, Patrobus is adapted to habitats that have been around for a long time, so I propose that they are among the older extant carabid species. Both Hopkins *et al.* (1971) and Matthews (1979a) found fossils which they tentatively identified as P. septentrionis, which were dated as pre-Pliocene. This indicates that at least one species of Patrobus has probably existed essentially unchanged for more than five million years.

In light of this information, it would be very interesting to re-examine the relationship between Patrobus and its sister genus, Platypatrobus. The single species of Platypatrobus, P. lacustris Darlington, is morphologically very similar to Patrobus. According to G. E. Ball (personal communication), Lindroth would have placed the species within Patrobus, except that he chose not to question the judgement of Darlington (1938), who proposed Platypatrobus as a genus. P. lacustris lives in and on beaver houses, a habitat that is not far removed, either spatially or ecologically, from the habitats of Patrobus species. Possibly it is simply a Patrobus that has developed adaptations for life in a specialized habitat, and thus has diverged somewhat from other Patrobus species. This could be tested in a detailed cladistic study.

Within Diplous, populations of D. aterrimus display a genetic identity measure in agreement with Thorpe's values for populations within a species. D. aterrimus and D. californicus are also separated by a level within Thorpe's range for species within a genus. Genetically, Patrobus is almost completely diverged from Diplous. Thus, the genera are probably very old.

6.5.2 Evolutionary Hypotheses Within Patrobus

Distance Phenogram

The distance analysis (Figure 6.1) shows that the branch lengths in the diagram fit the pairwise distances very closely, as shown by the cophenetic correlation value of 0.988. This means that there is very little homoplasy hypothesized in the phenogram. However, the high percent standard deviation and F-value show that branch points and lengths are not

all that certain. This should be kept in mind during examination of relationships in the phenogram.

The four populations of 'inland' P. fossifrons are very closely related in this figure, and as a group they are quite far away from their sister group, the 'coastal' P. fossifrons. The various populations of P. stygicus are also very close together. Because of the large standard deviation, branching patterns of populations within these taxa are probably not significant. Clearly the populations within these taxa are very close, and there is a significant difference between P. fossifrons and P. stygicus, as was established in previous chapters.

This phenogram suggests that the Patrobus (*sensu stricto*) species P. septentrionis is closer to P. stygicus than P. fossifrons is. This goes against the morphologically based hypothesis, which placed P. septentrionis and P. stygicus in different subgenera. As well, another member of Patrobus (*sensu stricto*), P. atrorufus, is placed closer to P. stygicus and P. fossifrons than P. lecontei and P. longicornis are. This also contradicts previous ideas, since P. fossifrons, P. stygicus, P. lecontei, and P. longicornis are thought to form the monophyletic subgenus Neopatrobus (Darlington 1938, Lindroth 1961).

Although this phenogram is the best fit to the pairwise distance data, it reflects only overall levels of similarity between the taxa. While many similarities between taxa reflect phylogenetic relationships, this is certainly not always so.

Discrete Character Phylogenies

The discrete character analysis should provide a truer reflection of relationships, since it is based on phylogenetic principles. Figures 6.2 and 6.3 are the phylogenies resulting from the alternative techniques of determining allele presence; the absolute criterion, and the 5% criterion, respectively. These phylogenies are basically the same, except that the one based on the 5% criterion places P. atrorufus and P. longicornis into a position which is only one of three suggested in the phylogeny based on the absolute criterion. Neither reconstruction places P. septentrionis with certainty, though in both reconstructions this species is placed in a trichotomy with P. foveocollis and P. stygicus. The high level of uncertainty regarding the placement of P. atrorufus, P. longicornis, and P. lecontei is probably due to the small samples. In this study, the 5% criterion is probably the more valid technique, due to these small sample sizes, so I accept it as the best discrete character phylogeny.

Comparison of Distance and Discrete Character Results

The distance and discrete phenograms do not agree. This is not entirely unexpected, as the distance technique does not provide an accurate reflection of evolution. Mickevich and

Johnson (1976) pointed out that phenetic techniques were susceptible to error if the characters had been evolving at different rates, but cladistic analyses take variable rates into account. Mickevich and Johnson compared electrophoretic and morphological data for Menidia fish species, and found that, although characters within the data sets were shown to be evolving at different rates, cladistic techniques still produced similarly branching trees. This reinforces the idea that cladistic studies are superior to phenetic ones. Thus, I feel that the discrete character phylogeny of Patrobus, despite containing some uncertainties, is the most feasible evolutionary hypothesis that this genetic data set can offer.

Comparison of Electrophoretic and Morphological Character Phylogenies

Regardless of the placement of P. septentrionis and P. lecontei in the electrophoretic-based phylogeny, there are several differences between that phylogeny and the morphologically-based phylogeny. P. fossifrons and P. stygicus are not sister taxa in the electrophoretic-based hypothesis, and P. atrorufus, P. septentrionis, and P. foveocollis each independently destroy the monophyly of the subgenus Neopatrobus. Since the reconstructions of Darlington (1938) and Kühnelt (1941) are not based on rigorous cladistic analysis of characters, the electrophoretic and morphological hypotheses cannot be compared by the rigorous methods of Mickevich and Johnson (1976). Instead, they must be compared more informally.

Both of the hypotheses cannot be correct. The electrophoretic hypothesis can be questioned, because the data suffer from the following problems. Some of the population samples are very small (under 10 specimens for four species), so they may not be representative of the species as a whole. Also, only a very small amount of the entire genome of these beetles was sampled, which may not have been a representative measure of their genetic make-up. These are serious problems, which cast doubt on the hypothesis. As well, the Nei's genetic identity measures (Table 6.1) suggest that perhaps the group is too diverged for electrophoresis to provide enough synapomorphies for an informative phylogenetic analysis.

The morphologically-based phylogeny reconstruction also could be incorrect. After all, it dates back prior to the development of rigorous cladistic methods, so taxa are not supported by specifically postulated synapomorphies. One could also argue that, like the electrophoretic study, it relies on a very small suite of characters, since it appears that Darlington and Kühnelt used primarily the male genitalia. These are very specialized structures which could have been under some unique evolutionary pressures, and thus may show trends counter to the direction of overall evolution. Another problem with the

morphologically-based hypothesis is that chorological affinities have played a significant part in determining relationships. Generally, it is good to use as many different characters as possible in generating a hypothesis. However, I feel that the distribution data were perhaps not characters so much as they were biases applied to the interpretation of the morphological characters, at a time when past geological and climatic changes were not well understood.

In spite of these problems, these two hypotheses could be the best interpretations of their respective data types. These two character systems are independent of one another, and are susceptible to different environmental pressures. The morphological characters could be very well-adapted to environmental conditions, and thus be both slow to change, and susceptible to parallel evolution of similar states. On the other hand, the electrophoretic data could be under no pressure to remain the same. They could have collected many mutations, to the point that many apparent synapomorphies between species are in fact homoplasies. If so, these differing rates of evolution would explain why the two data types yielded different phylogenies.

Other authors have reported that different characters can evolve at different rates. Turner (1974) compared morphologically-based and electrophoretically-based phenograms of five species of desert pupfish (Cyprinodon), which were very different morphologically, but were very similar electrophoretically. He reported that the two data sets produced very different phylogenies, which he concluded was due to different evolutionary rates. Maxson and Wilson (1974, 1975) compared morphological and immunological characters in frogs in the genus Hyla, and found marked differences. They wrote that two species which were very close morphologically were so far apart immunologically that they could be put into separate genera. They concluded that the immunological data were more accurate, and that the morphological similarity was due to convergence.

Because of different rates of evolution in different characters, the phenetic techniques employed in the above studies resulted in different phenograms. However, as Mickevich and Johnson (1976) pointed out, cladistic techniques should result in similar phylogenies from the different characters, because they are unaffected by different rates of evolution. This was not so in the Patrobus study, probably because of the small sample sizes.

Relationships in Patrobus

I am not prepared to offer any formal reconstructed phylogeny as the correct one. I do not think that the electrophoretically-based phylogeny is flawless, but I think it calls the old morphological ideas into question. Both could be wrong, but the fact that they are different means that a detailed study of the group is in order. Neither type of data is inherently

superior, so I suggest that any future cladistic analysis incorporate large suites of morphological, electrophoretic, and other types of characters.

Implications with regards to Species Concepts

If the Patrobus electrophoretic data are even partly correct, it is possible that P. fossifrons and P. stygicus are not sister taxa. However, it has been shown that they can hybridize, and produce viable offspring. Depending which species concept is followed, P. fossifrons and P. stygicus may or may not be separate species (see Chapter 5). If they are considered to be the same species, and if the electrophoretic-based phylogeny is correct, then three different subgenera of Patrobus would have to be collapsed into a single species, to form a monophyletic group including the hybridizing P. fossifrons and P. stygicus. However, many of these taxa are very different, and clearly deserve status as separate species.

I do not believe that ability to hybridize is a good reason for combining species, because it is a plesiotypic trait. Since the ability to hybridize depends on both the amount and the nature of genetic change occurring between the populations in question, it does not tell us very much about relationships. As a result, I feel that reproductive isolation does not make a good species concept.

Summary and Conclusion

In conclusion, the electrophoretically based and morphologically based reconstructed phylogenies of Patrobus do not concur. This is probably due to conservatism of morphological characters, and a high rate of evolution in genetic characters, leading to parallel evolution. Neither reconstruction is acceptable now, so it is recommended that a detailed cladistic study be done using large numbers of electrophoretic, morphological, and other characters.

6.6. Summary

Electrophoretic data from six North American species of Patrobus and one European species were examined. Pairwise distance data indicates that Patrobus is a very old group, which has diverged very little morphologically, but very much genetically. In light of this, it is suggested here that Platypatrobus lacustris, the sister group to Patrobus, may in fact be an aberrant Patrobus.

Phenograms resulting from distance analysis and discrete character analysis were compared. The reconstructed phylogeny based on discrete characters was less certain about

relationships, but it was deemed to be theoretically superior, because the methods used to produce it portray evolutionary theory more accurately. Thus the uncertainty in this analysis is deemed to be an indication of convergent evolution of electrophoretic characters. In addition, small population samples and the low number of loci sampled probably contributed to the uncertainty.

The discrete character phylogeny was compared to the accepted morphologically-based phylogeny, and was found to be quite different. It was suggested that this was due to different rates of evolution of electrophoretic and morphological characters. Neither phylogeny was accepted, as each is based on only one type of data. Depending which one is true, the hybridizing species P. fossifrons and P. stygicus may not be sister species, and the subgenus Neopatrobus may not be a monophyletic entity. It is suggested that a detailed revision of Patrobus be done, using several types of characters, to determine the true relationships among species.

Table 6.1: Pairwise genetic comparisons of Patrobus and Diplous population samples. Above diagonal = Nei's (1972) genetic identities; below diagonal = Cavalli-Sforza and Edwards (1967) arc distances.

SAMPLE:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
'inland' P. fossifrons:																				
1. Rock	----	.998	.998	.998	.617	.408	.415	.415	.402	.401	.405	.422	.421	.409	.342	.549	.448	.002	.000	.012
2. Tern	.114	----	.998	.997	.591	.400	.407	.407	.394	.394	.398	.414	.413	.420	.340	.546	.453	.002	.000	.011
3. Lynch	.078	.100	----	.998	.596	.407	.414	.414	.401	.400	.405	.421	.420	.422	.358	.564	.446	.002	.000	.015
4. Curlew	.080	.116	.046	----	.602	.401	.409	.409	.396	.395	.401	.415	.414	.415	.351	.559	.446	.002	.000	.015
'coastal' P. fossifrons:																				
5. Wauconda	.515	.583	.563	.562	----	.495	.481	.502	.491	.487	.487	.502	.489	.211	.240	.412	.387	.000	.000	.019
P. stygicus:																				
6. Lost Rd.	.784	.786	.784	.786	.731	----	.993	.998	.999	.998	.998	.994	.973	.166	.174	.323	.439	.004	.000	.000
7. Pecten	.766	.766	.766	.768	.747	.130	----	.991	.995	.995	.995	.988	.959	.161	.177	.352	.446	.007	.000	.000
8. Chain	.778	.781	.777	.778	.732	.077	.156	----	.997	.995	.997	.994	.976	.177	.177	.328	.454	.004	.000	.000
9. George	.782	.785	.782	.783	.728	.060	.130	.073	----	.999	.999	.993	.969	.165	.175	.324	.438	.004	.000	.000
10. Long	.795	.797	.794	.796	.733	.073	.145	.108	.074	----	.998	.994	.969	.171	.178	.334	.440	.004	.000	.000
11. Hinton	.782	.784	.779	.780	.741	.101	.150	.082	.076	.097	----	.993	.971	.170	.175	.335	.451	.004	.000	.001
12. Lynch	.792	.792	.792	.794	.735	.108	.185	.140	.139	.118	.149	----	.959	.170	.179	.333	.452	.006	.000	.000
13. Wauconda	.791	.794	.794	.796	.725	.174	.247	.167	.175	.185	.182	.198	----	.175	.172	.348	.464	.000	.000	.000
P. lecontei:																				
14. Lynch	.781	.777	.763	.778	.857	.863	.890	.856	.866	.862	.860	.872	.871	----	.556	.364	.465	.000	.000	.042
P. longicornis:																				
15. Edmonton	.805	.812	.806	.809	.835	.878	.892	.884	.881	.877	.893	.883	.891	.685	----	.535	.158	.000	.000	.094
P. atrorufus:																				
16. Finland	.638	.635	.630	.635	.732	.799	.787	.803	.796	.784	.791	.808	.798	.779	.698	----	.342	.009	.004	.068
P. septentrionis:																				
17. Hinton	.752	.749	.754	.753	.781	.744	.756	.735	.746	.748	.740	.737	.734	.729	.922	.797	----	.000	.000	.113
D. aterrimus:																				
18. Rocky Mt. House	.992	.992	.992	.992	1.0	.987	.982	.987	.987	.988	.987	.985	1.0	1.0	1.0	.979	1.0	----	.927	.507
19. Waterton	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	.989	1.0	.213	----	.472
D. californicus:																				
20. Similkameen	.997	.980	.971	.971	.975	.998	1.0	.996	.997	.998	.994	1.0	1.0	.947	.925	.945	.943	.683	.707	----

Table 6.2: Binary electrophoretic data codings Patrobus and Diplous species and morphs. '1' = presence and '0' = absence of alleles. Left column contains absolute presence / absence data; the right column uses the 5% criterion (f must be at least .05). Loci one to eight are *Ao*, *Apk*, *Est-1*, *Est-3*, *Hk*, *Odh*, *Pgi*, and *Pgm*, respectively.

	ABSOLUTE DATA - locus and allele:	5% DATA - locus and allele:
	11122333334444444556666666677777888888	11233333444444556666777788888
SPECIES / MORPH:	EFGCDABCEFBEGJKLMFGBCDEFHIKBCEGIABCDFGI	EFCABCEFBEGJKMFGDFHKBCEGCDFI
'inland' P. fossifrons	01110001100000101110000110010000110100 1	01100110000011100110100001000
'coastal' P. fossifrons	00110001100000111100000110010101000100 1	00100110000011100110101001010 1
P. stygicus	10011110010011010110101000010101010101 1	10111001000100100000001001000
P. lecontei	01011101011000000011000010001100000010 0	01110101100000010010011000100
P. longicornis	01010001100111000000011000001100000010 1	01100110011100001000011000110
P. foveocollis	10011000011000000010110000010000010000 0	10100001100000011000100000000
P. atrorufus	01010001100110000101000011110111000111 0	01100110011000100011101101100
P. septentrionis	10010100111000000100000010000010001000 0	10110011100000100010000110000
Diplous	00000011000000000000000001110000011010 0	00001100000000000001000010100

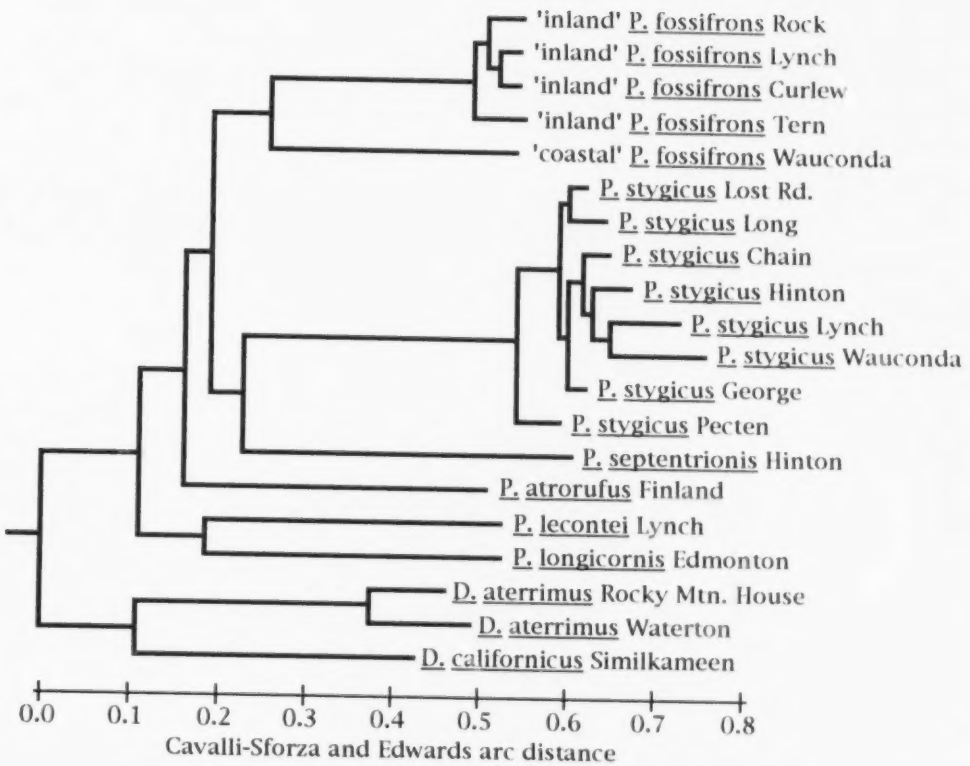


Figure 6.1: Phenogram of Patrobus and Diplous species and populations, based on Cavalli-Sforza and Edwards (1967) arc distances. Total length of tree = 4.247; cophenetic correlation = 0.988; Prager and Wilson's (1976) F value = 7.154; standard deviation = 10.869%.

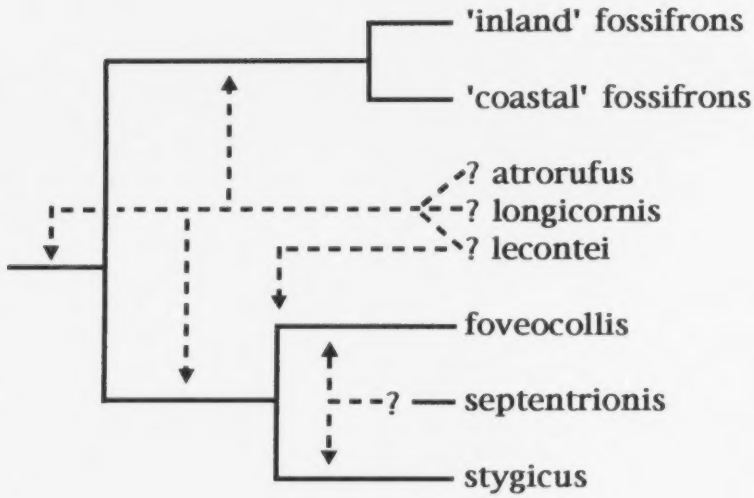


Figure 6.2: Reconstructed phylogeny of Patrobus species, using absolute presence/absence of alleles as binary character states. Dashed lines indicate uncertainty of placement of some taxa.

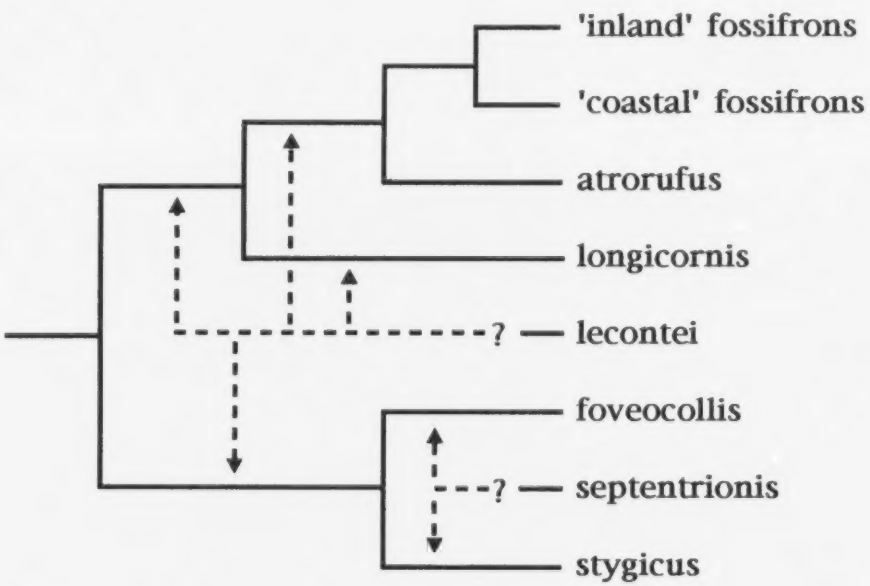


Figure 6.3: Reconstructed phylogeny of Patrobus species, using 5% criterion of presence/absence of alleles as binary character states. Dashed lines indicate uncertainty of placement of some taxa.

7.CONCLUSIONS

7.1 Answers to Questions

This project was started with four questions in mind, which I will now attempt to answer.

1. Is the current taxonomic status of P. stygicus and P. fossifrons correct?

P. stygicus and P. fossifrons are distinct in allopatry, and are more-or-less distinct in sympatry. Hybridization occurs in sympatry, but it is rare, and the resulting offspring are strongly selected against. Gene flow is low enough that P. stygicus and P. fossifrons remain distinct species. They cannot be distinguished by any one morphological character, but when several characters are considered, the vast majority can be identified. As well, fixed differences at three loci distinguish P. fossifrons and P. stygicus. They tend to live in different environments, and select different microhabitats at sympatric sites.

2. Is there any variation within P. fossifrons or P. stygicus which ought to be recognized taxonomically?

P. stygicus was homogeneous across all study sites, but P. fossifrons was divided into two morphs, here named 'coastal' and 'inland' morphs. These do not correspond to Darlington's (1938) P. fossifrons fossifrons and P. fossifrons dimorphicus. The 'coastal' morph of P. fossifrons lives along the Pacific coast, from Alaska to Washington, and east as far as the Crowsnest Pass. The 'inland' morph lives from central British Columbia to the Cypress Hills, and south into the northwestern United States. These morphs can usually be distinguished by slight morphological differences, and by a fixed difference at the *Ao* locus. They are quite distant electrophoretically, and gene flow between them is low enough that they are capable of diverging via genetic drift. However, they are not formally recognized taxonomically, because the nature of the geographical division between them is not known.

3. Does hybridization occur between P. fossifrons and P. stygicus? What conditions allow it to occur or prevent it from occurring?

Hybridization does occur between P. fossifrons and P. stygicus, so despite their differences in structure, biochemistry, and habitat, these taxa are not completely reproductively isolated from one another. This ability to hybridize is not an indicator of their relatedness, because they were found to be quite distant genetically.

Two putative hybrids of P. stygicus and 'inland' P. fossifrons (out of 299 examined specimens) were found at Pincher Creek. P. stygicus generally lives at higher elevations, in the boreal zone, and 'inland' P. fossifrons lives at lower elevations, on the prairie. However, the two zones overlap at Pincher Creek, as well as in the Cypress Hills. At these sites, P. stygicus and 'inland' P. fossifrons encounter one another, and occasionally interbreed. Resulting hybrids are rare, and if they are fertile, they are strongly selected against.

One P. stygicus / 'coastal' P. fossifrons hybrid was found (out of 30 examined specimens) at Wauconda, Washington. It was at least an F_2, so obviously some hybrids are fertile. There was no other evidence of mixed stock there, so hybrids must be very rare, and/or strongly selected against. Although P. stygicus generally lives farther north than 'coastal' P. fossifrons, they occurred in sympatry at Wauconda, with no evidence of habitat partitioning.

4. Are the currently accepted phylogenetic positions of P. fossifrons and P. stygicus within Patrobus correct?

A phylogeny reconstructed from electrophoretic data was quite different from the accepted morphologically-based phylogeny. Unlike the morphologically-based hypothesis, the electrophoretic-based hypothesis suggested that P. fossifrons and P. stygicus are not sister species, and that their subgenus, Neopatrobus, is not a monophyletic group. Neither of these phylogenies is accepted by me; I suggest that the group be re-examined, using many different types of characters.

7.2 General Conclusions

Although P. fossifrons and P. stygicus do not conform completely to the biological species concept, based on reproductive isolation, strong barriers are evident between them, so they are clearly distinct. The two morphs of P. fossifrons are not so diverged. They are different enough that they are capable of evolving in different directions, and will probably be much more distinct in the future.

This study is quite similar to that of Mossakowski *et al.* (1990), who examined hybridization in the carabid genus Chrysocarabus. They examined morphological and electrophoretic data, and found that hybrids were best identified by a suite of morphological characters. Although they found higher levels of hybridization (2 to 16%), they noted aberrant hind wings in hybrids, and an absence of the F_1 generation of hybrids, similar to

the current study. These facts, as well as the fact that males were not fertile, led them to conclude that the two taxa were not compatible enough to be classified as the same species.

These taxa show us that our attempts at applying a classification system of discrete units to a complex, changing world is not always successful. Instead of sharp boundaries, some species have grey areas between themselves and other taxa.

When I started this project, I attempted to go beyond the question "what?", to start asking "how?", and thus try to shed some light on "why?". I have described what P. fossifrons and P. stygicus are like, and have suggested some reasons as to how they have come to be like that. As for why they are the way they are, I have come to see species not as simple compartments in which to place living things, but rather as artificial constructs of ours which do not always describe life around us accurately.

The idea of "species" tends to hide the complex forces acting on living things. Evolutionary forces result in a clustering of individuals into similar types that we call "species", but this discrete labelling distances us from the fact that they are often only partially clustered. We need to be aware of the shortcomings of our classification system, and see the underlying forces causing variation among organisms, if we are to really understand the living world.

REFERENCES CITED

AYALA, F. J., POWELL, J. R., TRACEY, M. L., MOURAO, C., A., and PEREZ-SALAS, S. 1972. Enzyme variability in the Drosophila willistoni group. IV. Genic variation in natural populations of Drosophila willistoni. Genetics, 70:113-139.

BIÉMONT, C. 1983. Homeostasis, enzymatic heterozygosity and inbreeding depression in natural populations of Drosophila melanogaster. Genetica, 61:179-189.

BREITUNG, A. J. 1954. A botanical survey of the Cypress Hills. The Canadian Field Naturalist, 68:55-92.

BREWER, G. J. 1970. Specific electrophoretic systems. *In* An Introduction to Isozyme Techniques. *Edited by* G. J. Brewer and C. F. Sing. Academic Press, New York. pp. 62-137.

BUTH, D. G. 1984. The application of electrophoretic data to systematic studies. Annual Review of Ecology and Systematics, 15:501-522.

CAMPBELL, J. M., and PECK, S. B. 1990. Omalonomus relictus, an unusual new genus and new species (Coleoptera: Staphylinidae, Omaliinae) of blind rove beetle; a preglacial (Tertiary?) relict in the Cypress Hills, Alberta-Saskatchewan, Canada. The Canadian Entomologist, 122:949-961.

CARTER, A. 1971. Some aspects of the comparative ecology of populations of the four Patrobus species (Coleoptera: Carabidae: Patrobini) at George Lake, Alberta. M.Sc. Thesis, University of Alberta.

CAVALLI-SFORZA, L. L., and EDWARDS, A. W. F. 1967. Phylogenetic analysis: models and estimation procedures. Evolution, 21:550-570.

CROTHER, B. I. 1990. Is "some better than none" or do allele frequencies contain phylogenetically useful information? Cladistics, 6:277-281.

DARLINGTON, P. J. Jr. 1936 Variation and atrophy of flying wings of some carabid beetles. Annals of the Entomological Society of America, 49:136-179.

DARLINGTON, P. J. Jr. 1938. The American Patrobini (Coleoptera: Carabidae). Entomologica Americana, 18:135-173.

DEJEAN, C. P. F. M. A. 1821. Catalog de la collection des coléoptères de M. le Baron Dejean. Paris.

DEN BOER, P. J., VAN HUIZEN, T. H. P., DEN BOER-DAANJE, W., AUKEMA, B., and DEN BIEMAN, C. F. M. 1980. Wing polymorphism and dimorphism in ground beetles as stages of an evolutionary process (Coleoptera: Carabidae). Entomologia Generalis, 6:107-134.

FARRIS, J. S. 1981. Distance data in phylogenetic analysis. In Advances in Cladistics: Proceedings of the First Meeting of the Willi Hennig Society. Edited by V. A. Funk and D. R. Brooks. The New York Botanical Garden, Bronx, New York. pp. 3-23.

FELSENSTEIN, J. 1985. Confidence limits of phylogenies: an approach using the bootstrap. Evolution, 39:783-791.

FILDES, R. A., HARRIS, H. 1966. Genetically determined variation of adenylate kinase in man. Nature, 209:261.

FISHER, R. A. 1936. The use of multiple measurements in taxonomic problems. Annals of Eugenics, 7:179-188.

FITCH, W. M., and MARGOLIASH, E. 1967. Construction of phylogenetic trees. Science, 155:279-284.

GOODING, R. H., and ROLSETH, B. M. 1979. Genetics of Glossina morsitans morsitans (Diptera: Glossinidae) IV. Electrophoretic banding patterns of octanol dehydrogenase and arginine phosphokinase. The Canadian Entomologist, 111:1307-1310.

GOODING, R. H., and ROLSETH, B. M. 1982. Genetics of Glossina morsitans morsitans (Diptera: Glossinidae) VI. Multilocus comparison of three laboratory populations. Canadian Journal of Genetics and Cytology, 24:109-115.

GRAHAM, J. H., and FELLEY, J. D. 1985. Genomic coadaptation and developmental stability within introgressed populations of Enneacanthus gloriosus and E. obesus (Pisces: Centrarchidae). Evolution, 39:104-114.

HABU, A. 1972. On some Carabidae found by Dr. S.-I. Uéno in Hokkaido, North Japan. Mushi, 46:29-38.

HABU, A. 1973. Notes and descriptions of Formosan Carabidae taken by Dr. S.-I. Uéno in 1961 (Coleoptera: Carabidae) II. A new Broscosoma and two new Patrobus species. Transactions of the Shikoku Entomological Society, 11:99-106.

HABU, A. 1976. Three new species of Apatrobus, a subgenus of Patrobus, from Shikoku, Japan (Coleoptera: Carabidae). Transactions of the Shikoku Entomological Society, 13:9-17.

HALDANE, J. B. S. 1922. Sex ratio and unisexual sterility in hybrid animals. Journal of Genetics, 12:101-109.

HAMMOND, P. M. 1979. Wing-folding mechanisms of beetles, with special reference to investigations of adephagan phylogeny (Coleoptera). *In* Carabid Beetles: Their Evolution, Natural History, and Classification. *Edited by* T. L. Erwin, G. E. Ball, and D. R. Whitehead. Dr. W. Junk, The Hague. pp. 113-180.

HARRIS, H., and HOPKINSON, D. A. 1977. Handbook of Enzyme Electrophoresis in Human Genetics. Elsevier/North Holland Biochemical Press, Amsterdam.

HILLIS, D. M. 1984. Misuse and modification of Nei's genetic distance. Systematic Zoology, 33:238-240.

HOPKINS, D. M., MATTHEWS, J. V. Jr., WOLFE, J. A., and SILBERMAN, M. L. 1971. A Pliocene flora and insect fauna from Bering Strait region. Palaeography, Palaeoclimatology, Palaeoecology, 9:211-231.

HUBBS, C. L., and HUBBS, C. 1953. An improved graphical analysis and comparison of series of samples. Systematic Zoology, 2:49-56.

JOHNSON, A. G., UTTER, F. M., and HODGINS, H. O. 1970. Interspecific variation of tetrazolium oxidase in Sebastodes (rockfish). Comparative Biochemistry and Physiology, 37:281-285.

JOHNSON, G. B. 1977. Evaluation of the stepwise mutation model of electrophoretic mobility: comparison of the gel sieving behavior of alleles at the Esterase-5 locus of Drosophila pseudoobscura. Genetics, 87:139-157.

JOHNSON, G. B. 1978. Structural flexibility of isozyme variants: genetic variants on Drosophila disguised by cofactor and subunit binding. Proceedings of the National Academy of Sciences of the USA, 75:395-399.

KARLSTROM, T. N. V., and BALL, G. E., *editors*. 1969. The Kodiak Island Refugium: its Geology, Flora, Fauna, and History. Ryerson Press, Toronto.

KAT, P. W. 1982. The relationship between heterozygosity for enzyme loci and developmental homeostasis in peripheral populations of aquatic bivalves (Unionidae). American Naturalist, 119:824-832.

KAVANAUGH, D. H. 1979. Rates of taxonomically significant differentiation in relation to geographical isolation and habitat: examples from a study of the nearctic Nebria fauna. *In* Carabid Beetles: Their Evolution, Natural History, and Classification. *Edited by* T. L. Erwin, G. E. Ball, and D. R. Whitehead. Dr. W. Junk, The Hague. pp. 35-57.

KAVANAUGH, D. H. 1985. On wing atrophy in carabid beetles (Coleoptera: Carabidae), with special reference to nearctic Nebria. *In* Taxonomy, Phylogeny, and Zoogeography of Beetles and Ants. *Edited by* G. E. Ball. Dr. W. Junk, Dordrecht. pp. 408-431.

KAVANAUGH, D. H. 1988. The insect fauna of the Pacific Northwest coast of North America: present patterns and affinities and their origins. *In* Origins of the North American Insect Fauna. *Edited by* J. A. Downes and D. H. Kavanaugh. Memoirs of the Entomological Society of Canada, No. 144. pp. 125-150.

KIMURA, M. 1953. "Stepping-stone" model of population. Annual Report of the National Institute of Genetics of Japan, 3:62-63.

KING, M. C., and WILSON, A. C. 1975. Evolution at two levels. Molecular similarities and biological differences between humans and chimpanzees. Science, 188:107-116.

KLUGE, A. G., and FARRIS, J. S. 1969. Quantitative phyletics and the evolution of anurans. Systematic Zoology, 18:1-32.

KRISCH, K. 1971. Carboxylic ester hydrolases. *In* The Enzymes, Volume 5. *Edited by* P. Boyer. Academic Press, New York. pp. 43-69.

KRYZHANOVSKIY, O. L. 1976. An attempt at a revised classification of the family Carabidae (Coleoptera). Entomological Review, 55:56-64.

KÜHNELT, W. 1941. Revision der Laufkäfer-gattungen Patrobus und Diplous. Annalen des Naturhistorischen Museums in Wien, 51:151-192.

LANGOR, D. W., and SPENCE, J. R. 1991. Host effects on allozyme and morphological variation of the mountain pine beetle, Dendroctonus ponderosae Hopkins (Coleoptera: Scolytidae). The Canadian Entomologist, 123:395-410.

LEARY, R. F., ALLENDORF, F. W., and KNUDSON, R. L. 1983. Developmental stability and enzyme heterozygosity in rainbow trout. Nature, 301:71-72.

LEARY, R. F., ALLENDORF, F. W., and KNUDSON, R. L. 1984. Superior developmental stability of heterozygotes of enzyme loci in salmonid fishes. American Naturalist, 124:540-551.

LEARY, R. F., ALLENDORF, F. W., and KNUDSON, R. L. 1985a. Inheritance of meristic variation and the evolution of developmental stability in rainbow trout. Evolution, 39:308-314.

LEARY, R. F., ALLENDORF, F. W., and KNUDSON, R. L. 1985b. Developmental instability and high meristic counts in interspecific hybrids of salmonid fishes. Evolution, 39:1318-1326.

LEDOUX, G. 1984. Nouveaux Penetretus du Cachemire Indien (Coléoptères, Patrobinae). Societe Entomologique de France Annales (N. S.), 20:399-409.

LERNER, I. M. 1954. Genetic Homeostasis. Wiley, New York.

LINDROTH, C. H. 1946. Inheritance of wing dimorphism in Pterostichus anthracinus Ill.. Hereditas, 32:37-40.

LINDROTH, C. H. 1961. The ground-beetles of Canada and Alaska, Part 2. Opuscula Entomologica, Suppl. 20.

LINDROTH, C. H. 1974. Handbooks for the identification of British insects, Volume 4, Part 2: Coleoptera: Carabidae. Royal Entomological Society of London, London, UK.

LINDROTH, C. H. 1979. The theory of glacial refugia. *In* Carabid Beetles: Their Evolution, Natural History, and Classification. *Edited by* T. L. Erwin, G. E. Ball, and D. R. Whitehead. Dr. W. Junk, The Hague. pp. 385-394.

MATTHEWS, J. V. Jr. 1979a. Late Tertiary carabid fossils from Alaska and the Canadian archipelago. *In* Carabid Beetles: Their Evolution, Natural History, and Classification. *Edited by* T. L. Erwin, G. E. Ball, and D. R. Whitehead. Dr. W. Junk, The Hague. pp. 425-445.

MATTHEWS, J. V. Jr. 1979b. Tertiary and Quaternary environments: historical background for an analysis of the Canadian insect fauna. *In* Canada and its Insect Fauna. *Edited by* H. V. Danks. Memoirs of the Entomological Society of Canada, No. 108. pp. 31-86.

MAXSON, L. R. and WILSON, A. C. 1974. Convergent morphological evolution detected by studying proteins of tree frogs in the Hyla eximia species group. Science, 185:66-68.

MAXSON, L. R., and WILSON, A. C. 1975. Albumin evolution and organismal evolution in tree frogs (Hylidae). Systematic Zoology, 24:1-15.

MAY, B., MARSDEN, J. E., and SCHENK, C. G. Unpublished. Electrophoretic procedures, recipes, and nomenclature used in the Cornell laboratory for ecological and evolutionary genetics.

MAYR, E. 1940. Speciation phenomena in birds. The American Naturalist, 74:249-278.

MAYR, E. 1969. Principles of Systematic Zoology. McGraw-Hill, Toronto.

MICKEVICH, M. F., and JOHNSON, M. S. 1976. Congruence between morphological and allozyme data in evolutionary inference and character evolution. Systematic Zoology, 25:260-270.

MICKEVICH, M. F., and MITTER, C. 1981. Treating polymorphic characters in systematics: A phylogenetic treatment of electrophoretic data. *In* Advances in Cladistics: Proceedings of the First Meeting of the Willi Hennig Society. *Edited by* V. A. Funk and D. R. Brooks. The New York Botanical Garden, Bronx, New York. pp.45-58.

MICKEVICH, M. F., and MITTER, C. 1982. Evolutionary patterns in allozyme data: a systematic approach. *In* Advances in Cladistics: Proceedings of the Second Meeting of the Willi Hennig Society. *Edited by* N. I. Platnick and V. A. Funk. Columbia University Press, New York. pp.169-176.

MOSSAKOWSKI, D., ROSCHEN, A., and VAJE, S. 1986. Hybridization in Chrysocarabus. *In* Carabid Beetles: Their Adaptations and Dynamics. *Edited by* P. J. den Boer, M. L. Luff, D. Mossakowski, and F. Weber. Gustav Fischer, Stuttgart, pp. 281-295.

MOSSAKOWSKI, D., BRAUN, S., and ROSCHEN, A. 1990. Hybridization in natural populations of ground beetles (Coleoptera: Carabidae). The Canadian Journal of Zoology, 68:1783-1789.

MUNROE, E. 1956. Canada as an environment for insect life. The Canadian Entomologist, 88:372-476.

MURPHY, R. W., SITES, J. W. Jr., BUTH, D. G., and HAUFLER, C. H. 1990. Proteins I: Isozyme Electrophoresis. *In* Molecular Systematics. *Edited by* D. M. Hillis and C. Moritz. Sinauer Associates, Sunderland, MA. pp. 44-126.

NAKANE, T. 1978. The beetles of Japan (N. S.) 52. Harpalidae 6. Nature Insects, 13(6):9-12.

NEI, M. 1972. Genetic distance between populations. The American Naturalist, 106:283-292.

NEI, M. 1975. Molecular Population Genetics and Evolution. American Elsevier, New York.

NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics, 89:583-590.

NELSON, G. J., and PLATNICK, N. I. 1981. Systematics and Biogeography: Cladistics and Vicariance. Columbia University Press, New York.

NICHOLS, E., CHAPMAN, V. M., and RUDDLE, H. F. 1973. Polymorphism and linkage for mannosephosphate isomerase in Mus musculus. Biochemical Genetics, 8:47-53.

NOMENCLATURE COMMITTEE OF THE INTERNATIONAL UNION OF BIOCHEMISTRY. 1984. Enzyme Nomenclature. Academic Press, Orlando, Florida.

OHTA, T., and KIMURA, M. 1973. A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. Genetical Research, 22:201-204.

PALMER, A. R., and STROBECK, C. 1986. Fluctuating asymmetry: measurement, analysis, patterns. Annual Review of Ecology and Systematics, 17:391-421.

PATERSON, H. E. H. 1978. More evidence against speciation by reinforcement. South African Journal of Science, 74:369-371.

PATERSON, H. E. H. 1985. The recognition concept of speciation. *In* Species and Speciation. *Edited by* E. S. Vrba. Transvaal Museum Monograph No. 4, Transvaal Museum, Pretoria. pp. 21-29.

POHL, G. R., Unpublished (Entomology 401 project paper for G. E. Ball, 1987). Morphological variation in Patrobus fossifrons and P. stygicus (Carabidae: Patrobini) with consideration of possible hybrid populations.

PRAGER, E. M., and WILSON, J. M. 1976. Congruency of phylogenies derived from different proteins. Journal of Molecular Evolution, 9:45-57.

RICHARDSON, R. H., and SMOUSE, P. E. 1976. Patterns of molecular variation. I. Interspecific comparisons of electromorphs in the Drosophila mulleri complex. Biochemical Genetics, 14:447-466.

RICHARDSON, R. H., SMOUSE, P. E., and RICHARDSON, M. E. 1977. Patterns of molecular variation. II. Associations of electrophoretic mobility and larval substrate within species of the Drosophila mulleri complex. Genetics, 85:141-154.

ROGERS, J. S. 1972. Measures of genetic similarity and genetic distance. *In* Studies in Genetics VII. *Edited by* M. R. Wheeler. University of Texas Publication #7213, Austin, Texas. pp. 145-153.

ROLSETH, B. M., and GOODING, R. H. 1978. Genetics of Glossina morsitans morsitans (Diptera: Glossinidae) I. Electrophoretic banding patterns of xanthine oxidase and aldehyde oxidase. The Canadian Entomologist, 110:1233-1239.

SCHWARTZ, M. K., NISSELBAUM, J. S., and BODANSKY, O. 1963. Procedure for staining zones of activity of glutamic oxaloacetic transaminase following electrophoresis with starch gel. American Journal of Clinical Pathology, 40:103-106.

SELANDER, R. K. 1976. Genic variation in natural populations. *In* Molecular Evolution. *Edited by* F. J. Ayala. Sinauer Associates, Sunderland, MA. pp.21-45.

SHAW, C. R., and PRASAD, R. 1970. Starch gel electrophoresis of enzymes - a compilation of recipes. Biochemical Genetics, 4:297-320.

SIMPSON, G. G. 1961. Principles of Animal Taxonomy. Columbia University Press, New York.

SLATKIN, M. 1985a. Rare alleles as indicators of gene flow. Evolution, 39:53-65.

SLATKIN, M. 1985b. Gene flow in natural populations. Annual Review of Ecology and Systematics, 16:393-430.

SOKAL, R. R., and ROHLF, F. J. 1981. Biometry, Second Edition. W. H. Freeman, San Francisco.

SOKAL, R. R., and CROVELLO, T. J. 1970. The biological species concept: a critical evaluation. The American Naturalist, 104:127-153.

SOLTIS, D. E., HAUFLER, C. H., DARROW, D. C., and GASTONY, G. J. 1983. Starch gel electrophoresis of ferns: a compilation of grinding buffers, gel and electrode buffers, and staining schedules. American Fern Journal, 73:9-27.

SPERLING, F. A. H. 1987. Evolution of the Papilio machaon species group in western Canada (Lepidoptera: Papilionidae). Quaestiones Entomologicae, 23:198-315.

SWOFFORD, D. L. 1981. On the utility of the distance Wagner procedure. *In* Advances in Cladistics: Proceedings of the First Meeting of the Willi Hennig Society. *Edited by* V. A. Funk and D. R. Brooks. The New York Botanical Garden, Bronx, New York. pp.25-43.

SWOFFORD, D. L., and BERLOCHER, S. H. 1987. Inferring evolutionary trees from gene frequency data under the principle of maximum parsimony. Systematic Zoology, 36:293-325.

SWOFFORD, D. L., and OLSEN, G. J. 1990. Phylogeny reconstruction. *In* Molecular Systematics. *Edited by* D. M. Hillis and C. Moritz. Sinauer Associates, Sunderland, MA. pp. 411-501.

SWOFFORD, D. L., and SELANDER, R. B. 1981. BIOSYS-1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. Journal of Heredity, 72:281-283.

TEMPLETON, A. R. 1989. The meaning of species and speciation: a genetic perspective. *In* Speciation and its Consequences. *Edited by* D. Otte and J. A. Endler. Sinauer Associates Inc., Sunderland, MA.

THORPE, J. P. 1979. Enzyme variation and taxonomy: the estimation of sampling errors in measurements of interspecific genetic similarity. Biological Journal of the Linnean Society, 11:369-386.

THORPE, J. P. 1983. Enzyme variation, genetic distance, and evolutionary divergence in relation to levels of taxonomic separation. *In* Protein Polymorphism: Adaptive and Taxonomic Significance. *Edited by* G. S. Oxford and D. Rollinson. Academic Press, New York. pp. 131-152.

TURNER, B. J. 1974. Genetic divergence of Death Valley pupfish species: biochemical versus morphological evidence. Evolution, 28:281-294.

WARD, R. D. 1979. Metathoracic wing structures as phylogenetic indicators in the Adephaga (Coleoptera). *In* Carabid Beetles: Their Evolution, Natural History, and Classification. *Edited by* T. L. Erwin, G. E. Ball, and D. R. Whitehead. Dr. W. Junk, The Hague. pp. 181-191.

WRIGHT, S. 1931. Evolution in mendelian populations. Genetics, 16:97-159.

WRIGHT, S. 1951. The genetical structure of populations. Annals of Eugenics, 15:323-354.

WRIGHT, S. 1978. Evolution and the Genetics of Populations: Volume 4; Variability In and Among Natural Populations. The University of Chicago Press, Chicago.

APPENDIX #1. COLLECTION LOCALITY DATA

Table A1.1: Collection data of sampled populations of Patrobus and Diplous. 'MORPH.' and 'ELECTRO.' columns contain numbers of females (F), males (M), or specimens of unknown gender (?) sampled for morphological and electrophoretic characters, respectively.

CODE	COLLECTION LOCALITY	COLLECTION DATE	TOTAL F	TOTAL M	TOTAL (?)	MORPH. F	MORPH. M	MORPH. (?)	ELECTRO. F	ELECTRO. M	ELECTRO. (?)	COLLECTOR	MUSEUM
P. fossifrons (inland)													
1A2	Alta., vic. Pincher Ck., Rock Pond 49°21'N 113°53'W	VI-29-89	33	37		33	37		29	24		GR Pohl	UASM
1B2	Alta., vic. Pincher Ck., Tern Pond 49°22'N 113°43'W	VI-29-89	12	8		12	8		12	7		GR Pohl	UASM
1C3	Alta., vic. Pincher Ck., Lynch Lks. 49°23'N 113°57'W	VII-01-89	10	10		10	10		10	10		GR Pohl	UASM
1C4	Alta., vic. Pincher Ck., Lynch Lks. 49°23'N 113°57'W	VII-20-89	8	9		8	9		7	9		GR Pohl	UASM
1E1	Alta., vic. Pincher Ck., Bull Pond #1 49°21'N 113°54'W	VII-09-87	0	2		0	2		0	0		GR Pohl	UASM
1F1	Alta., Porcupine Hills, Pothole Pond 49°40'N 113°52'W	VII-07-87	3	7		3	7		0	0		GR Pohl	GR Pohl
1F2	Alta., Porcupine Hills, Pothole Pond 49°40'N 113°52'W	VI-07-89	1	0		1	0		1	0		GR Pohl	GR Pohl
1G1	Alta., 6 mi. s. Pincher Ck., Kavanaugh's Pond	VII-18-70	7	17		7	17		0	0		Kavanaugh	CAS
1H1	Idaho, 40 mi. n. Arco	VII-30-60	4	9		4	9		0	0		GE Ball et al.	UASM
1I1	Montana, Beaverhead Co., jct. US Rte. 91 & Big Hole R.	IX-04-66	4	3		4	3		0	0		GE Ball et al.	UASM
1J1	Alta., Cypress Hills Park, Elkwater Lake	VII-03-57	0	2		0	2		0	0		GE Ball et al.	UASM
1K1	Alta., 11 mi. s. Pincher Ck.	VII-26-56	11	25		11	25		0	0		GE Ball	UASM
1L2	Alta., Porcupine Hills, Curlew Pond 49°41'N 113°52'W	VII-11-88	3	2		3	2		3	2		GR Pohl	UASM
4C1	Alta., Del Bonita	VI-10-55	1	0		1	0		0	0		GE Ball	UASM
P. fossifrons (coastal)													
2A2	B.C., Rte. 3, 2 km w. Manning Park, Manning Pond	VII-13-89	1	0		1	0		1	0		GR Pohl	UASM
2B2	B.C., Rte. 3, 16 km n. Yahk, Deep Pond	VI-13-89	0	1		0	1		0	1		GR Pohl	UASM
2C2	B.C., Rte. 3, 24 km n. Yahk, Train Pond	VI-13-89	0	1		0	1		0	1		GR Pohl	UASM
2D1	B.C., Creston	VI-19-58	0	1		0	1		0	0		GE Ball	UASM
1D2	Wash., Okanagan Co., Wauconda Pond 48°40'N 119°00'W	VII-18-89	11	9		11	9		11	7		GR Pohl	UASM
2F1	B.C., St. Mary's Lake nr. Kimberley	VIII-27-55	6	2		6	2		0	0		GE Ball et al.	UASM
2G1	Alta., Crowsnest Pass, lake 1 mi w. Crowsnest Lake	1955-56	2	15		2	15		0	0		GE Ball	UASM
2H1	B.C., Vancouver I., vic. Victoria	1955-58	~~44~~	38		~~44~~	38		0	0		GE Ball et al.	UASM
2I1	Alaska, Kodiak I.	1958-62	12	10		12	10		0	0		GE Ball	UASM

Table A1.1, continued.

P. stygicus													
3A2	Alta., vic. Pincher Ck., Lost Rd. Pond 49°17'N 113°57'W	VI-28-89	22	29		22	29		19	28		GR Pohl	UASM
3A3	Alta., vic. Pincher Ck., Lost Rd. Pond 49°17'N 113°57'W	VII-01-89	4	16		4	16		3	15		GR Pohl	UASM
3B2	Alta., vic. Pincher Ck., Pecten Pond 49°18'N 113°58'W	VI-30-89	4	4		4	4		4	4		GR Pohl	UASM
3C2	Alta., Waterton Park, Blakiston Ck. 49°06'N 113°55'W	VI-28-89	0	1		0	1		0	1		GR Pohl	UASM
3D2	Alta., vic. Pincher Ck., Antenna Pond 49°19'N 113°58'W	VII-01-89	0	1		0	1		0	1		GR Pohl	UASM
3E1	Alta., Livingstone Mts., Chain Ponds 49°42'N 114°18'W	VII-11-87	2	5		2	5		0	0		GR Pohl	GR Pohl
3E2	Alta., Livingstone Mts., Chain Ponds 49°42'N 114°18'W	VII-14-88	0	1		0	1		0	1		GR Pohl	UASM
3E3	Alta., Livingstone Mts., Chain Ponds 49°42'N 114°18'W	VI-11-89	14	11		13	10		14	11		GR Pohl	UASM
3E4	Alta., Livingstone Mts., Chain Ponds 49°42'N 114°18'W	VII-01-89	12	16		12	16		12	15		GR Pohl	UASM
3E5	Alta., Livingstone Mts., Chain Ponds 49°42'N 114°18'W	VIII-22-89	12	6		12	6		12	6		GR Pohl	UASM
3F3	Alta., George Lake Entom. Field Stn. 53°55'N 114°05'W	VIII-15-88	9	8		3	2		8	8		GR Pohl	UASM
3F5	Alta., George Lake Entom. Field Stn. 53°55'N 114°05'W	V-31-89	3	6		1	0		2	6		GR Pohl	UASM
3F6	Alta., George Lake Entom. Field Stn. 53°55'N 114°05'W	VI-20-89	7	7	(7)	7	6	(3)	7	7	(7)	GR Pohl	UASM
3F7	Alta., George Lake Entom. Field Stn. 53°55'N 114°05'W	VII-06-89	19	38		19	38		16	36		GR Pohl	UASM
3F8	Alta., George Lake Entom. Field Stn. 53°55'N 114°05'W	VII-20-89	0	1		0	1		0	1		H Nychka	UASM
3F9	Alta., George Lake Entom. Field Stn. 53°55'N 114°05'W	VIII-09-90	2	1		2	1		2	1		GR Pohl	UASM
3G2	Alta., Rte. 831, Long Lake Pond 54°25'N 112°50'W	VI-22-88	8	9		4	4		4	5		GR Pohl	UASM
3G3	Alta., Rte. 831, Long Lake Pond 54°25'N 112°50'W	VII-27-88	8	16		4	9		8	16		GR Pohl	UASM
3G4	Alta., Rte. 831, Long Lake Pond 54°25'N 112°50'W	VIII-11-88	5	10	(1)	0	0	(1)	5	10	(1)	GR Pohl	UASM
3G5	Alta., Rte. 831, Long Lake Pond 54°25'N 112°50'W	VII-07-89	4	7		4	7		4	7		GR Pohl	UASM
3H2	Alta., 30 km n. Hinton, Dave's Pond	VII-11-89	5	4		5	4		5	4		DW Langor	UASM
3H3	Alta., 30 km n. Hinton, Dave's Pond	VII-31-89	8	8		8	8		8	8		GR Pohl	UASM
3I2	Alta., vic. Pincher Ck., Lynch Lks. 49°23'N 113°57'W	VII-01-89	2	5		2	5		2	5		GR Pohl	UASM
3I3	Alta., vic. Pincher Ck., Lynch Lks. 49°23'N 113°57'W	VII-20-89	2	0		2	0		2	0		GR Pohl	UASM
3J2	Wash., Okanagan Co., Wauconda Pond 48°40'N 119°00'W	VII-18-89	3	6		3	6		3	6		GR Pohl	UASM
3K1	Alaska, Circle	VII-3+4-58	16	26		16	26		0	0		GE Ball	UASM
3L1	B.C., n. end Williams Lake	VI-05-58	10	15		10	15		0	0		GE Ball	UASM
3N1	Alta./Sask., Cypress Hills Park	1955-64	17	27		17	27		0	0		GE Ball et al.	UASM
3O1	B.C., Cranbrook	1956+1960	30	28		30	28		0	0		GE Ball et al.	UASM
3P1	B.C., s. end Columbia Lake, Canal Flats	VII-31-56	6	8		6	8		0	0		GE Ball	UASM
3Q1	B.C., 11 mi. e. Wardner	VII-27-56	4	2		4	2		0	0		GE Ball	UASM
3R1	Alta., 6 mi. s. Pincher Ck., Kavanaugh's Pond	VII-18-70	10	8		10	8		0	0		Kavanaugh	CAS
3S1	Alta., Kananaskis Lake	VI-02-69	1	3		1	3		0	0		D Larson	BDUC
3T1	Alta., vic. Pincher Ck., Bull Pond #2 49°21'N 133°55'W	VII-12-88	1	0		1	0		0	0		GR Pohl	UASM
3V1	B.C., 35 mi. n. Kimberley, Island Pond	VIII-28-55	0	1		0	1		0	0		GE Ball et al.	UASM

Table A1.1, continued.

P. stygicus/fossifrons hybrids															
4A2	Wash., Okanagan Co., Wauconda Pond 48°40'N 119°00'W	VII-18-89	1	0		1	0		1	0				GR Pohl	UASM
4D2	Alta., vic. Pincher Ck., Rock Pond 49°21'N 113°53'W	VI-29-89	0	1		0	1		0	0				GR Pohl	UASM
4E1	Alta., 11 mi. s. Pincher Ck, Ball's Pond	VII-26-56	0	1		0	1		0	0				GE Ball	UASM
P. lecontei															
5A2	Alta., vic. Pincher Ck., Lynch Lks. 49°23'N 113°57'W	VII-01-89	3	2	(2)	3	2	(2)	3	2	(2)			GR Pohl	UASM
5A3	Alta., vic. Pincher Ck., Lynch Lks. 49°23'N 113°57'W	VII-20-89	0	0	(2)	0	0	(2)	0	0	(2)			GR Pohl	UASM
5C2	Alta., Porcupine Hills, Curlew Pond 49°41'N 113°52'W	VII-11-88	1	0		1	0		1	0				GR Pohl	UASM
5D2	Alta., George Lake Entom. Field Stn. 53°55'N 114°05'W	VII-19-88	1	0		1	0		1	0				GR Pohl	UASM
5E2	Alta., vic. Pincher Ck., Tern Pond 49°22'N 113°43'W	VI-29-89	0	1		0	1		0	1				GR Pohl	UASM
5F2	Alta., vic. Pincher Ck., Rock Pond 49°21'N 113°53'W	VI-29-89	1	2		1	2		1	2				GR Pohl	UASM
5G2	Alta., vic. Pincher Ck., Bennett Pond 49°13'N 113°46'W	VI-30-89	1	0		1	0		1	0				GR Pohl	UASM
5H2	Alta., vic. Pincher Ck., Pecten Pond 49°18'N 113°58'W	VI-30-89	1	0		1	0		1	0				GR Pohl	UASM
5I2	Alta., vic. Pincher Ck., Frith Pond 49°16'N 113°47'W	VI-27-89	0	1		0	1		0	0				GR Pohl	UASM
5J1	Alta., 6 mi. s. Pincher Ck., Kavanaugh's Pond	VII-18-70	0	1		0	1		0	0				Kavanaugh	CAS
5K1	Alta., Porcupine Hills, Pothole Pond 49°40'N 113°52'W	VII-07-87	1	0	0	1	0	0	0	0	0			GR Pohl	GR Pohl
P. longicornis															
11A1	Alta., Edmonton, N. Sask. R. valley nr. Big Island	VI+IX-85	4	6		4	6		0	0				GR Pohl	GR Pohl
11A2	Alta., Edmonton, N. Sask. R. valley nr. Big Island	VIII-08-90	5	4		5	4		5	4				GR Pohl	UASM
P. foveocollis															
8A2	Alta., 30 km n. Hinton, Dave's Pond	VII-11-89	1	0		1	0		1	0				DW Langor	UASM
8B2	Alta., Rte. 831, Long Lake Pond 54°25'N 112°50'W	VIII-11-88	1	0		1	0		1	0				GR Pohl	UASM
8C1	Alta., Kananaskis	V+VI-69	2	0		2	0		2	0				D Larson	BDUC
P. septentrionis															
7A2	Alta., 30 km n. Hinton, Dave's Pond	VII-11-89	0	1		0	1		0	1				DW Langor	UASM
7A3	Alta., 30 km n. Hinton, Dave's Pond	VII-31-89	1	5		1	5		1	4				GR Pohl	UASM
P. atrorufus															
6A2	Finland, vic. Helsinki, Vantaa, damp meadow, pit trap	V to VIII-89	4	6	(1)	4	6	(1)	4	5				JK Niemela	UASM
D. aterrimus															
9A2	Alta., N. Saskatchewan R., nr. Rocky Mountain House	VI-14-89	11	19	(4)	0	2		11	17	(4)			GR Pohl	UASM
9A3	Alta., N. Saskatchewan R., nr. Rocky Mountain House	VII-03-89	12	17		12	17		10	10				GR Pohl	UASM
9B2	Alta., Waterton R., 1 km s. Waterton Dam	VI-27-89	3	3		3	3		3	3				GR Pohl	UASM
9C2	Alta., Oldman R., at jct. Oldman R. & Rte. 785	VI-27-89	0	1		0	1		0	1				GR Pohl	UASM
D. californicus															
10A2	B.C., Similkameen R., 10 km w. Hedley	VI-13-89	15	26		15	26		15	18				GR Pohl	UASM

APPENDIX #2. CHEMICAL SUPPLIERS

Table A2.1: Chemicals used in polyacrylamide gel electrophoresis, and their suppliers.

COMPOUND	SUPPLIER
acetic acid	Fisher Scientific Co.
acrylamide	Schwarz/Mann Biotech Co.
adenosine-5'-triphosphate	Calbiochem Co.
ammonium persulfate	Fisher Scientific Co.
benzaldehyde	Fisher Scientific Co.
bromophenol blue	Fisher Scientific Co.
cellulose acetate papers	Gelman Sciences Inc.
DL-dithiothreitol	Sigma Chemical Co.
fast blue RR salt	Sigma Chemical Co.
D-fructose-6-phosphate	Sigma Chemical Co.
glucose	Fisher Scientific Co.
α-D-glucose-1-phosphate	Sigma Chemical Co.
glucose-6-phosphate dehydrogenase	Sigma Chemical Co.
glycine	Sigma Chemical Co.
hydrochloric acid	Fisher Scientific Co.
magnesium chloride	Fisher Scientific Co.
N,N'-methylene-bis-acrylamide	Schwarz/Mann Biotech Co.
α-naphthyl acetate	Sigma Chemical Co.
ß-nicotinamide adenine dinucleotide	Sigma Chemical Co.
ß-nicotinamide adenine dinucleotide phosphate	Sigma Chemical Co.
nitro blue tetrazolium	Sigma Chemical Co.
1-octanol	Sigma Chemical Co.
phenazine methosulfate	Sigma Chemical Co.
85% phosphoric acid	Fisher Scientific Co.
polyvinylpyrrolidone	Sigma Chemical Co.
riboflavin	Eastman Kodak Co.
sodium phosphate dibasic	Fisher Scientific Co.
sodium phosphate monobasic	Fisher Scientific Co.
N,N,N',N'-tetramethylethylenediamine	Eastman Kodak Co.
Trizma® R base	Sigma Chemical Co.
Trizma® R hydrochloride	Sigma Chemical Co.

APPENDIX #3. DETAILS OF ENZYME STAINS

Details of Successful Enzyme Staining Procedures

Aldehyde Oxidase; AO

The AO stain, modified from Rolseth and Gooding (1978), contained 2 drops benzaldehyde, 9mg nitro blue tetrazolium (NBT), and 3mg phenazine methosulfate (PMS) in 25ml 50mM tris buffer, pH 7.2. Bands became visible after being incubated at 37°C for about two minutes, and were readable for fresh and refrozen head-thoraces and abdomens on a 7% gel, at pH 8.2 or 8.9.

Arginine Phosphokinase; APK

Bands representing APK appeared on all gels when staining for other enzymes. With PMS-containing stains, APK appeared as a red band, as a result of direct binding with PMS (Gooding and Rolseth 1979). With esterase stains, APK appeared as a faint yellow band which faded after about 30 minutes. This was probably a result of the enzyme binding to the fast blue RR of the esterase staining solution. These yellow bands were clearly APK, since electromorphs always corresponded exactly to the PMS-stained APK electromorphs of the same specimen on other gels.

Esterases; EST

The EST stain was based on Sperling (personal communication), as modified from Gooding and Rolseth (1982) and Brewer (1970). To stain for esterases, the gel was soaked in 30ml of 200mM phosphate buffer, pH 6.0, at room temperature for seven minutes. Then a solution of 35mg of α-naphthyl acetate in 1.5ml acetone was added to the soaking gel, which was then incubated at 37°C for seven minutes. The gel was then put into a filtered mixture of 35mg α-naphthyl acetate in 1.5ml acetone, and 40mg fast blue RR salt in 30ml 200mM phosphate buffer, pH 6.0. Stained bands appeared within five minutes, at room temperature.

Several series of esterase bands appeared on 7% and 9% gels at pH 8.9, from fresh and refrozen head-thoracic and abdominal samples. Two of these provided readable information: EST-1 was clearest with 30μl of abdominal homogenate on a 9% gel, while EST-3 was clearest with 40μl of abdominal homogenate on a 7% gel. At least three other esterases were present (EST-2, -4, and -5), but due to their complex patterns, they could not be interpreted.

Hexokinase; HK

Rolseth's (personal communication) HK stain, a modification of Brewer (1970), was done with a cellulose acetate overlay. The staining solution contained 10mg glucose, 3mg NBT, 1mg PMS, 3mg ß-nicotinamide adenine dinucleotide phosphate (NADP), 1mg $MgCl_2 \cdot 6H_2O$, 5mg adenosine-5'-triphosphate (ATP), and 25μl of glucose-6-phosphate dehydrogenase (G-6-PD) in 3ml 200mM tris, pH 8.0. A small strip of cellulose acetate was soaked in this staining solution, blotted dry, and then laid onto the gel, which was incubated at 37°C until bands appeared. Results were obtained for frozen thoracic samples on 7% and 9% gels, pH 8.2 and 8.9. Best results were obtained with 30μl of head-thoracic homogenate on a 9% gel, pH 8.9.

Octanol Dehydrogenase; ODH

The ODH staining procedure was described originally by Ayala *et al.* (1972). The stain used in this study was modified from Gooding and Rolseth (1979), and contained 200μl 1-octanol, 9mg ß-nicotinamide adenine dinucleotide (NAD), 9mg NBT, and 3mg PMS in 25ml 50mM tris, pH 7.2. Bands appeared after incubation at 37°C for about 30 minutes. Enzymes were active with fresh and frozen head-thoracic and abdominal samples on 7% and 9% gels of pH 8.2 and 8.9. Best results were obtained with 40μl of abdominal homogenate, on a 7% gel, pH 8.2.

Phosphoglucose isomerase; PGI

Rolseth's (personal communication) stain for PGI, modified from Shaw and Prasad (1970), was used on a cellulose acetate overlay. The staining solution contained 10mg fructose-6-phosphate, 3mg NBT, 1mg PMS, 3mg NADP, 1mg $MgCl_2 \cdot 6H_2O$, and 25μl G-6-PD in 3ml 50mM tris, pH 7.2. The overlay procedure was the same as that for HK. Bands appeared after about two minutes at room temperature. Activity was seen in fresh and frozen head-thoracic and abdominal samples on 7% gels, pH 8.2. Best results were with 40μl of abdominal sample.

Phosphoglucomutase; PGM

The PGM staining technique was based on Rolseth's (personal communication) cellulose acetate modification of Shaw and Prasad's (1970) recipe. The staining solution contained 10mg glucose-1-phosphate, 1mg PMS, 3mg NADP, 1mg $MgCl_2 \cdot 6H_2O$, and 25μl G-6-PD in 3ml 50mM tris, pH 7.2. The overlay procedure was the same as that for HK. Bands appeared after about five minutes of incubation at 37°C. The enzyme was active in fresh and frozen head-thoracic and abdominal samples on 7% gels, pH 8.2 and 8.9. Best results were with 40μl of abdominal homogenate on 7% gels, pH 8.2.

Unsuccessful Enzyme Stains

Table A3.1: Enzyme stains attempted unsuccessfully on polyacrylamide gels. EC numbers are from Nomenclature Committee of the International Union of Biochemistry (1984).

ENZYME (REFERENCE)	ABBREVIATION
adenylate kinase (Fildes and Harris 1966)	(AK, EC 2.7.4.3)
alcohol dehydrogenase (May *et al.* Unpubl.)	(ADH)
catalase (Harris and Hopkinson 1977)	(Cat)
esterases (Brewer 1970)	(EST-2, -4, -5, EC 3.1.1.2)
general protein (Rolseth, Pers. comm.)	(GP)
glucose-6-phosphate dehydrogenase (Gooding and Rolseth 1982)	(G-6-PD, EC 5.3.1.8)
glutamate-oxaloacetate transaminase (Schwartz *et al.* 1963)	(GOT, EC 2.6.1.1)
α-glycerophosphate dehydrogenase (Gooding and Rolseth 1982)	(a-GPDH)
hydroxybutyrate dehydrogenase Shaw and Prasad (1970)	(HbDH)
isocitrate dehydrogenase (May *et al.* Unpubl.)	(IDH, EC 1.1.1.42)
lactic acid dehydrogenase (Shaw and Prasad 1970)	(LDH, EC 1.1.1.27)
malate dehydrogenase (Brewer 1970)	(MDH, EC 1.1.1.37)
malic enzyme (Ayala *et al.* 1972)	(ME, EC 1.1.1.40)
6-phosphogluconate dehydrogenase (Shaw and Prasad 1970)	(6-PGD, EC 1.1.1.44)
phosphomannose isomerase (Nichols *et al.* 1973)	(PMI, EC 5.3.1.8)
shikimic acid dehydrogenase (Soltis *et al.* 1983)	(SkDH)
sorbitol dehydrogenase (Shaw and Prasad 1970)	(SoDH, EC 1.1.1.14)
superoxide dismutase (Johnson *et al.* 1970)	(SOD)
xanthine oxidase (Gooding and Rolseth 1979)	(XO, EC 1.2.3.2)

APPENDIX #4. ELECTROPHORETIC DATA

Table A4.1: Electromorph codes of individual beetles, for the eight loci under study. Dashes indicate missing data.

SAMPLE, CODE & SEX	*Ao*	*Apk*	*Est-1*	*Est-3*	*Hk*	*Odh*	*Pgi*	*Pgm*
P. fossifrons (inland)								
Rock Pond, Alta. VI-29-1989								
1A2-2F	FF	CC	CE	KM	FF	FH	BB	DD
1A2-3F	FF	CC	EE	MM	FF	FH	BB	DD
1A2-5F	FF	CC	CE	MM	FF	HH	BB	DD
1A2-6F	FF	CC	CE	KM	FF	FH	BB	DD
1A2-7F	FF	CC	CE	KM	FF	HH	BB	DD
1A2-8F	FG	CC	EE	KK	FF	HH	BB	DD
1A2-9F	FF	CC	EE	KM	FF	HH	BB	DD
1A2-10F	FF	CC	EE	KK	FF	HH	BB	DD
1A2-11F	FF	CC	EE	KM	FF	FH	BB	DD
1A2-12F	FF	CC	EE	KK	FF	HH	BB	DD
1A2-13F	FF	CC	CC	KM	FF	FH	BB	DD
1A2-14F	FF	CC	EE	KK	FF	HH	BB	DD
1A2-15F	FF	CC	EE	KM	FF	HH	BB	DD
1A2-16F	FF	CC	EE	KM	FF	HH	BB	DD
1A2-17F	FF	CC	EE	KM	FF	HH	BB	DD
1A2-18F	FF	CC	CC	KK	FF	HH	BB	DD
1A2-19F	FF	CC	EE	KM	FF	HH	BB	DD
1A2-20F	FF	CC	EE	KK	FF	HH	BB	DD
1A2-21F	FF	CC	EE	KM	FF	HH	BB	DD
1A2-22F	FF	CC	EE	MM	FF	HH	BB	DD
1A2-23F	FF	CC	EE	KK	FF	HH	BB	DD
1A2-24F	FF	CC	CE	KM	FF	HH	BB	DD
1A2-25F	FF	CC	CE	MM	FF	HH	BB	DD
1A2-26F	FF	CC	EE	KK	FF	HH	BB	DD
1A2-44F	FF	--	EE	--	--	HH	BB	DD
1A2-45F	FF	--	CE	--	--	HH	BB	II
1A2-46F	FF	CC	EE	KK	--	HH	BB	DD
1A2-47F	FF	--	CC	--	--	FH	BB	--
1A2-48F	FF	--	EE	--	--	HH	BB	DI
1A2-1M	FF	CC	CE	KM	FF	FH	BB	DD
1A2-4M	FF	CC	EE	KK	FF	HH	BB	DD
1A2-27M	FF	CC	EE	KK	FF	HH	BB	DD
1A2-28M	FF	CC	EE	KK	FF	HH	BB	DD
1A2-29M	FF	CC	EE	MM	FF	HH	BB	DD
1A2-30M	FF	CC	CE	KM	FF	HH	BB	DD
1A2-31M	FF	CC	EE	KK	FF	HH	BB	BD
1A2-32M	FF	CC	CE	KK	FF	FH	BB	DD
1A2-33M	FF	CC	EE	MM	FF	HH	BB	DD
1A2-34M	FG	CC	EE	KK	FF	HH	BB	DD
1A2-35M	FF	CC	CE	KM	FF	HH	BB	DD
1A2-36M	FF	CC	EE	KM	FF	HH	BB	DD
1A2-37M	FF	CC	CE	KK	FF	HH	BB	DD
1A2-38M	FF	CC	EE	KK	--	HH	BB	DD
1A2-39M	FF	CC	EE	KK	--	HH	BB	DD
1A2-40M	FF	CC	EE	MM	FF	HH	BB	DD
1A2-41M	GG	CC	EE	KK	FF	HH	BB	DD
1A2-42M	FF	CC	EE	KM	--	HH	BB	DD
1A2-43M	FG	CC	EE	KM	FF	FH	BB	DD
1A2-49M	FF	--	EE	MM	--	HH	BB	BD
1A2-50M	FF	--	EE	KK	--	FH	BB	DD
1A2-51M	FF	CC	EE	KK	--	HH	BB	DD
1A2-52M	FF	--	EE	KM	--	HH	BB	DI
1A2-53M	FF	--	EE	--	--	HH	BB	DD

Table A4.1, continued.

Tern Pond, Alta.								
VI-29-1989								
1B2-7F	FF	CC	EE	KK	FF	HH	BB	DD
1B2-8F	--	CC	EE	KM	FF	HH	BB	DD
1B2-10F	FF	CC	CE	KM	FF	HH	BB	DD
1B2-11F	FF	CC	EE	KK	FF	HH	BB	DD
1B2-12F	FF	CC	EE	KK	FF	HH	BB	DD
1B2-13F	FF	CC	EE	KM	FF	HH	BB	DD
1B2-14F	FF	CC	CE	KM	FF	HH	BB	DD
1B2-15F	--	CC	EE	MM	FF	HH	BB	DD
1B2-16F	FF	CC	EE	KM	FF	HH	BB	DD
1B2-17F	FF	CC	EE	KM	FF	HH	BB	AA
1B2-18F	FF	CC	EE	KK	FF	HH	BB	DD
1B2-19F	--	CC	EE	KM	FF	HH	BB	DD
1B2-1M	--	CC	CE	KK	FF	HH	BB	DD
1B2-2M	--	CC	CE	KK	FF	HH	BB	DD
1B2-3M	FF	CC	EE	KM	FF	HH	BB	DD
1B2-4M	FF	CC	EE	KM	FF	HH	BB	DD
1B2-5M	FF	CC	CC	MM	FF	HH	BB	DD
1B2-6M	FF	CC	EE	KK	FF	HH	BB	DD
1B2-9M	FF	CC	EE	KM	FF	HH	BB	DD
Lynch Lakes, Alta.								
VII-01-1989								
1C3-1F	FF	CC	CE	KM	FF	FH	BB	DD
1C3-2F	FF	CC	CE	KK	FF	HH	BB	DD
1C3-3F	FF	CC	EE	KM	FF	HH	BB	DD
1C3-4F	FF	CC	CC	KM	FF	HH	BB	DD
1C3-5F	FF	CC	CE	KM	FF	HH	BB	DD
1C3-6F	FF	CC	EE	KM	FF	FH	BB	DD
1C3-7F	FF	CC	EE	KK	FF	HH	BB	DD
1C3-8F	FF	CC	CE	KK	FF	FH	BB	DD
1C3-11F	FF	CC	CE	KK	FF	HH	BB	DD
1C3-12F	FF	CC	EE	KK	FF	HH	BB	DD
1C3-13M	FF	CC	CE	KM	FG	HH	BB	DD
1C3-14M	FF	CC	EE	MM	FF	FH	BB	DD
1C3-15M	FF	CC	EE	KK	FF	HH	BB	DD
1C3-16M	FF	CC	EE	KK	--	HH	BB	DD
1C3-17M	FF	CC	CE	MM	FF	HH	BB	DD
1C3-18M	FF	CC	CE	KM	FF	HH	BB	DD
1C3-19M	FF	CC	CE	KK	FF	HH	BB	DD
1C3-20M	FF	CC	CE	KK	FF	HH	BB	DD
1C3-21M	FF	CC	EE	MM	FF	HH	BB	DD
1C3-22M	FF	CC	CE	KM	FF	HH	BB	DD
VII-20-1989								
1C4-1F	FF	CC	EE	MM	FF	HH	BB	DD
1C4-2F	FF	CC	EE	KM	FF	HH	BB	DD
1C4-5F	FF	CC	--	KM	FF	HH	BB	BD
1C4-6F	FF	CC	EE	KK	FF	HH	BB	DD
1C4-7F	FF	CC	EE	KK	FF	HH	BB	DD
1C4-9F	FF	CC	CE	KM	FF	HH	BB	BD
1C4-12F	FF	CC	EE	KK	FF	HH	BB	DD
1C4-3M	FF	CC	EE	MM	FF	HH	BB	DD
1C4-4M	FF	CC	CE	MM	FF	HH	BB	DD
1C4-8M	FF	CC	CE	KM	FF	HH	BB	DD
1C4-10M	FF	CC	EE	KM	FF	HH	BB	DD
1C4-11M	FF	CC	--	KK	FF	HH	BB	DD
1C4-14M	FF	CC	EE	KK	FF	HH	BB	DD
1C4-16M	FF	CC	--	KM	FF	HH	BB	BD
1C4-18M	FF	CC	CE	KK	FF	FH	BB	DD
1C4-19M	FF	CC	EE	KM	FF	HH	BB	DD
Pothole Pond, Alta.								
VI-07-1989								
1F2-1F	FF	CC	CE	KM	FF	HH	BB	DD

Table A4.1, continued.

Curlew Pond, Alta.								
VII-11-1988								
1L2-1F	FF	CC	EE	KM	FF	HH	BB	BD
1L2-3F	FF	CC	EE	KM	FF	FH	BB	DD
1L2-5F	FF	CC	CE	KK	FF	HH	BB	DD
1L2-2M	FF	CC	CE	KK	FF	HH	BB	DD
1L2-4M	FF	CC	EE	KM	FF	HH	BB	DD
P. fossifrons (coastal)								
Manning, B.C.								
VII-13-1989								
2A2-1F	GG	BB	EE	IK	FF	HH	BB	II
Deep Pond, B.C.								
VI-13-1989								
2B2-1M	GG	CC	EE	KK	FF	FH	BE	DI
Train Pond, B.C.								
VI-13-1989								
2C2-1M	GG	CC	CC	LL	FF	FH	EE	DI
Wauconda, Washington								
VII-18-1989								
1D2-1F	GG	CC	EE	KK	FF	HH	EE	DD
1D2-2F	GG	CC	EE	KM	FF	FF	EE	DD
1D2-4F	GG	CC	EE	KK	FF	HH	EE	DI
1D2-5F	GG	CC	EE	KM	FF	FF	EE	DI
1D2-7F	GG	CC	EE	MM	FF	HH	EI	II
1D2-9F	GG	CC	CE	KM	FF	FF	EE	II
1D2-11F	GG	CC	EE	KM	FF	FF	EE	II
1D2-14F	GG	CC	CE	KK	FF	FF	EE	DI
1D2-16F	GG	CC	CC	KK	FF	HH	EI	DI
1D2-23F	GG	CC	EE	MM	FF	FF	EE	II
1D2-25F	GG	CC	EE	KM	FF	FH	EE	IK
1D2-6M	GG	CC	CE	KK	FF	FF	EE	DI
1D2-8M	GG	CC	CE	MM	FF	FF	EE	DI
1D2-10M	GG	CC	CE	MM	FF	FF	EE	II
1D2-13M	GG	CC	EE	MM	FF	FF	EE	DD
1D2-17M	GG	CC	EE	KM	FF	FH	EE	IK
1D2-21M	GG	CC	EE	KK	FF	FH	EE	DI
1D2-26M	GG	CC	CC	KK	FF	FH	EE	DI
P. stygicus								
Lost Rd. Pond, Alta.								
VI-28-1989								
3A2-1F	EE	CC	AB	JJ	FF	GG	EE	DD
3A2-2F	EE	CC	AA	JJ	FF	GG	EE	DD
3A2-3F	EE	CC	AB	JJ	FF	GG	EE	DD
3A2-4F	EE	CC	AA	JJ	FF	GG	EE	DD
3A2-5F	EE	CC	AA	JJ	FF	GG	BE	DD
3A2-6F	--	CC	AA	JJ	FF	GG	EE	DD
3A2-7F	--	CC	AB	JJ	FF	GG	EE	DD
3A2-8F	EE	CC	AA	JJ	FF	GG	EE	DD
3A2-9F	EE	CC	AB	JJ	FF	EG	EE	DD
3A2-10F	EE	CC	AB	JJ	FF	EG	EE	DI
3A2-11F	EE	CC	AB	JJ	FF	GG	EE	DD
3A2-12F	EE	CC	AA	JJ	FF	GG	EE	DD
3A2-13F	EE	CC	AA	JJ	FF	GG	EE	DD
3A2-14F	EE	CC	AA	JJ	FF	GG	EE	DD
3A2-15F	EE	CC	AA	JJ	FF	GG	EE	DD
3A2-16F	EE	CC	AA	JL	FF	GG	EE	DD
3A2-46F	EE	CC	AA	JL	FF	GG	EE	DD
3A2-47F	EE	CC	AA	JJ	FG	GG	EE	DG
3A2-48F	EE	CC	AA	JJ	FF	GG	EE	DD
3A2-18M	EE	CC	AB	JJ	FF	GG	EE	DD
3A2-19M	EE	CC	AA	JJ	FF	GG	EE	DD

Table A4.1, continued.

3A2-20M	EE	CC	AA	JL	FF	GG	EE	DD
3A2-21M	EE	CC	AA	JL	FF	GG	EE	DD
3A2-22M	EE	CC	AA	JJ	FF	GG	EE	DD
3A2-23M	EE	CC	AF	GJ	FF	GG	EE	DG
3A2-24M	EE	CC	AA	JJ	FF	GG	EE	DD
3A2-25M	EE	CC	AF	JJ	FF	GG	EE	DD
3A2-26M	EE	CC	AA	JL	FF	GG	EE	DD
3A2-27M	EE	CC	AF	JJ	FF	GG	EE	DD
3A2-28M	EE	CC	AA	JJ	FF	GG	EE	D-
3A2-29M	--	CC	AA	JL	FF	GG	EE	DD
3A2-30M	EE	CC	BB	JJ	FF	GG	EE	DD
3A2-31M	EE	CC	AA	JL	FF	GG	EE	DD
3A2-32M	--	CC	AA	JL	FF	GG	EE	DD
3A2-33M	EE	CC	AA	JJ	FF	GG	EE	DD
3A2-34M	--	CC	AA	JJ	FF	GG	EE	DD
3A2-35M	EE	CC	AA	JL	FG	GG	EE	BD
3A2-36M	EE	CC	BB	JJ	FF	GG	EE	DD
3A2-37M	EE	CC	AA	JJ	FF	GG	EE	DD
3A2-38M	EE	CC	AA	JJ	FF	GG	EE	DD
3A2-39M	EE	CC	AA	LL	FF	GG	EE	DD
3A2-40M	EE	CC	AA	JJ	FF	GG	EE	DD
3A2-41M	EE	CC	AA	JJ	FF	GG	EE	DD
3A2-42M	EE	CC	AA	JJ	FF	EG	EE	DD
3A2-43M	EE	CC	AA	JJ	FF	GG	EE	DD
3A2-44M	EE	CC	AA	JL	FF	GG	EE	DD
3A2-45M	EE	CC	BF	JJ	FF	GG	EE	DD
VII-01-1989								
3A3-1F	EE	CC	AA	JJ	FF	GG	EE	DD
3A3-2F	EE	CC	AB	GJ	FF	GG	EE	DD
3A3-3F	EE	CC	AA	JJ	FF	GG	EE	DD
3A3-4M	EE	CC	AA	JJ	FF	GG	EE	DD
3A3-5M	EE	CC	AA	JJ	FF	GG	EE	DD
3A3-6M	EE	CC	AA	JL	FF	GG	EE	DD
3A3-7M	--	CC	AA	JL	FG	GG	EE	DD
3A3-8M	--	CC	AA	JJ	FF	GG	EE	DD
3A3-9M	--	CC	AB	JJ	FF	GG	EE	DD
3A3-10M	--	CC	AA	JJ	FF	GG	EE	DD
3A3-11M	EE	CC	AA	JJ	FF	GG	EI	DD
3A3-12M	EE	CC	AB	LL	FF	GG	EE	DD
3A3-13M	EE	CD	AA	JJ	FF	GG	EE	DD
3A3-14M	EE	CC	AB	JJ	FF	GG	EE	DD
3A3-15M	--	CC	AA	JJ	FF	GG	EE	DD
3A3-16M	EE	CC	AA	JJ	FF	GG	EE	DI
3A3-17M	--	CC	AA	JJ	FF	GG	EE	DD
3A3-18M	EE	CC	AF	JJ	FF	GG	EE	DD
Pecten Pond, Alta.								
VI-30-1989								
3B2-1F	EE	CC	AB	JJ	FF	GG	EE	DD
3B2-2F	EE	CC	AA	JJ	FF	GG	EE	DG
3B2-3F	EE	CC	AA	JJ	FF	GG	EE	DD
3B2-4F	EE	CC	AA	JJ	FF	GG	EE	DD
3B2-5M	EE	CC	AB	JJ	FF	GG	BE	DD
3B2-6M	EE	CC	AA	JJ	FF	GG	BE	DD
3B2-7M	EE	CC	AA	JJ	FF	GG	EE	DD
3B2-8M	EE	CC	AB	JL	FF	GG	EE	GG
Blakiston Ck., Alta.								
VI-28-1989								
3C2-1M	EE	CC	AA	JL	FF	GG	EE	DD
Antenna Pond, Alta.								
VII-01-1989								
3D2-1M	EE	CC	AA	JL	FF	GG	EE	DD

Table A4.1, continued.

Chain Ponds, Alta.								
VII-14-1988								
3E2-1M	EE	CC	BB	JJ	FF	GG	EE	DD
VI-11-1989								
3E3-1F	EE	CC	AB	JJ	FF	GG	EE	DD
3E3-2F	EE	CC	AB	JJ	FF	GG	EE	DD
3E3-3F	EE	CC	AA	JL	FF	GG	EE	DD
3E3-4F	EE	CC	AA	JJ	FF	GG	EE	DD
3E3-5F	EE	CC	AF	JL	FF	GG	EE	DD
3E3-6F	EE	CC	AB	JJ	FF	GG	EE	DD
3E3-7F	EE	CC	AA	JJ	FF	GG	EE	DD
3E3-8F	EE	CC	BB	JL	FF	GG	EE	DD
3E3-9F	EE	CC	AA	JJ	FF	GG	EE	DD
3E3-10F	EE	CC	AB	JJ	FF	GG	EE	DI
3E3-11F	EE	CC	AA	JJ	FF	GG	EE	DD
3E3-12F	EE	CC	FF	JJ	FF	CG	EE	DD
3E3-102F	EE	--	AA	JJ	--	GG	EE	--
3E3-103F	EE	CC	AF	--	--	GG	EE	DD
3E3-13M	EE	CC	AA	JL	FF	GG	EE	DD
3E3-14M	EE	CC	AA	JJ	FF	GG	EE	DD
3E3-15M	EE	CC	AA	JL	FF	GG	EE	DD
3E3-16M	EE	CC	AA	JL	FF	CG	EE	DD
3E3-17M	EE	CC	AA	JJ	FF	GG	EE	DD
3E3-18M	EE	CC	AA	JJ	FF	GG	BE	DD
3E3-19M	EE	CC	AF	JJ	FF	GG	EE	DD
3E3-20M	EE	CC	AF	JJ	FF	GG	EE	DD
3E3-21M	EE	CC	AA	JJ	FF	GG	EE	--
3E3-101M	EE	--	BB	JJ	--	GG	EE	--
3E3-104M	EE	CC	AA	JJ	--	GG	EE	DD
VII-01-1989								
3E4-16F	--	CC	AA	JJ	--	GG	EE	DD
3E4-17F	EE	CC	AA	JL	FF	GG	EE	DD
3E4-18F	--	CC	AA	JJ	--	GG	EE	--
3E4-19F	--	CC	AA	JJ	--	GG	EE	DD
3E4-20F	--	CC	AA	JJ	--	GG	EE	DD
3E4-21F	EE	CC	AF	JL	FF	GG	EE	DD
3E4-22F	--	CC	AF	JL	--	CG	EE	DD
3E4-23F	--	CC	AA	LL	FF	GG	EE	DD
3E4-24F	EE	CC	AA	JJ	FF	GG	EE	DD
3E4-25F	EE	CC	AF	JJ	FF	GG	EE	DD
3E4-26F	EE	CC	AA	JJ	FF	GG	EI	DD
3E4-27F	--	CC	AA	JL	FF	CG	EE	DD
3E4-1M	--	CC	AA	JL	FG	GG	EE	DD
3E4-2M	EE	CC	AA	JJ	FF	GG	EE	--
3E4-3M	--	CC	AF	JJ	FF	GG	EE	DI
3E4-4M	--	CC	AA	JL	FF	GG	EE	DD
3E4-5M	EE	CC	AA	JJ	FF	GG	BE	DD
3E4-6M	EE	CC	AA	LL	FF	GG	EE	DD
3E4-7M	EE	CC	AA	JJ	FF	GG	EE	DD
3E4-8M	EE	CC	AF	JJ	FF	GG	EE	DD
3E4-9M	EE	CC	AA	JJ	FF	GG	EE	DD
3E4-10M	EE	CC	AB	JJ	FF	GG	EE	DD
3E4-11M	EE	CC	AF	JL	FF	GG	EE	DD
3E4-12M	EE	CC	AA	JJ	FF	GG	EE	DG
3E4-13M	EE	CC	AA	JJ	FF	GG	EE	DD
3E4-14M	EE	CC	BF	JJ	FF	GG	EE	DD
3E4-15M	EE	CC	AA	JJ	FF	GG	EE	DD
VIII-22-1989								
3E5-1F	EE	CC	AA	JJ	FF	GG	EE	BD
3E5-2F	EE	CC	AF	LL	FF	GG	EE	DD
3E5-3F	EE	CC	AB	JJ	FF	GG	EE	DD
3E5-5F	EE	CC	AF	JJ	FG	GG	EE	DD
3E5-6F	EE	CC	BF	JJ	FF	GG	EE	DD

Table A4.1, continued.

3E5-7F	EE	CC	AB	GL	FG	GG	EE	DD
3E5-8F	EE	CC	AB	JL	FF	GG	EE	DD
3E5-9F	EE	CC	AF	JJ	FF	GG	EE	BB
3E5-11F	EE	CC	AA	JJ	FF	GG	EE	DD
3E5-13F	EE	CC	AA	JJ	FF	GG	EE	DD
3E5-14F	EE	CC	AA	JJ	FF	GG	EE	DD
3E5-15F	EE	CC	AA	JJ	FF	GG	EE	DD
3E5-4M	EE	CC	AA	JJ	FF	GG	EE	BD
3E5-10M	EE	CC	AA	JJ	FF	GG	BE	DD
3E5-12M	EE	CC	AF	JJ	FF	GG	EE	DD
3E5-16M	EE	CC	AF	JJ	FF	GG	EE	BD
3E5-17M	EE	CC	AF	LL	FF	GG	EE	DD
3E5-18M	EE	CC	AA	JL	FF	GG	EE	DD
George Lake, Alta.								
VIII-15-1988								
3F3-4F	EE	--	--	JJ	--	GG	--	--
3F3-7F	--	--	--	--	--	GG	--	--
3F3-15F	EE	--	--	JJ	--	GG	--	--
3F3-17F	EE	--	--	--	--	GG	EE	--
3F3-19F	--	--	--	--	--	--	EE	--
3F3-22F	EE	--	--	--	--	GG	EE	--
3F3-24F	--	--	--	--	--	GG	--	--
3F3-25F	--	--	--	--	--	--	EE	--
3F3-1M	--	--	--	--	--	--	EE	--
3F3-2M	EE	--	--	JJ	--	GG	--	--
3F3-5M	EE	--	--	JL	--	GG	--	--
3F3-8M	--	--	--	--	--	GG	--	--
3F3-10M	--	--	--	--	--	GG	--	--
3F3-13M	EE	--	--	--	--	GG	EE	--
3F3-16M	--	--	--	--	--	--	EE	--
3F3-18M	EE	--	--	--	--	GG	EE	--
V-31-1989								
3F5-4F	--	--	AA	JJ	--	--	--	--
3F5-8F	EE	--	--	--	--	--	--	DD
3F5-1M	--	--	AA	JJ	--	--	--	--
3F5-2M	--	--	--	JJ	--	--	--	--
3F5-3M	--	--	AA	JJ	--	--	--	--
3F5-5M	EE	--	--	--	--	--	--	DD
3F5-6M	EE	--	--	--	--	--	--	DD
3F5-7M	EE	--	--	--	--	--	--	DD
VI-20-1989								
3F6-4F	EE	CC	--	JJ	--	GG	EE	DD
3F6-5F	EE	CC	--	JJ	--	GG	EE	DD
3F6-6F	EE	CC	--	JL	--	GG	EE	DI
3F6-14F	EE	CC	--	JJ	--	GG	EE	--
3F6-18F	EE	CD	--	JJ	--	GG	EE	--
3F6-19F	EE	CC	--	JJ	--	GG	EE	--
3F6-21F	EE	CC	--	JJ	--	GG	EE	--
3F6-2M	EE	CC	--	JJ	--	GG	EE	DG
3F6-3M	EE	CC	--	JJ	--	GG	EE	DD
3F6-13M	EE	CC	--	JJ	--	GG	EE	--
3F6-15M	EE	CC	--	JJ	--	GG	EE	--
3F6-16M	EE	CC	--	JJ	--	GG	EE	--
3F6-20M	EE	CC	--	JJ	--	GG	EE	--
3F6-22M	EE	CC	--	JJ	--	GG	EE	--
3F6-1-	EE	CC	--	--	--	--	EE	DD
3F6-7-	EE	CC	AA	JJ	--	GG	EE	DD
3F6-8-	EE	CC	AA	JJ	--	GG	EE	DD
3F6-10-	EE	CC	--	--	--	--	EE	DD
3F6-11-	EE	CC	--	--	--	--	EE	DD
3F6-12-	EE	CC	--	--	--	--	EE	DD
3F6-17-	EE	CC	--	JL	--	GG	EE	--

Table A4.1, continued.

VII-06-1989								
3F7-38F	EE	CC	AA	JJ	FF	GG	EE	DD
3F7-39F	EE	CC	AA	JL	FF	GG	EE	DD
3F7-41F	EE	CC	AA	JJ	FF	GG	EE	DD
3F7-44F	EE	CC	AA	JJ	FF	GG	EE	DD
3F7-45F	EE	CC	AA	JJ	FG	GG	EI	DI
3F7-46F	EE	CC	AB	JL	FG	GG	EE	DD
3F7-47F	EE	CC	AA	JJ	FF	GG	EE	DD
3F7-48F	EE	CC	AA	GJ	FF	GG	EE	DD
3F7-49F	EE	CC	AF	JJ	FF	GG	EE	DD
3F7-50F	EE	CC	AB	JJ	FF	GG	EE	DD
3F7-51F	EE	CC	AA	JJ	FF	GG	EE	DD
3F7-52F	EE	CC	AA	JJ	FF	GG	EE	DD
3F7-53F	EE	CC	AA	JJ	FF	CG	EE	BD
3F7-54F	EE	CC	AA	JL	FF	GG	EE	DI
3F7-55F	EE	CC	AA	JJ	FF	GG	EE	DD
3F7-56F	EE	CC	AA	JJ	FF	GG	EE	DD
3F7-1M	EE	CC	AA	JL	--	GG	EE	DD
3F7-2M	EE	CC	AB	JJ	FF	GG	EE	DD
3F7-3M	EE	CC	AA	JJ	--	GG	BE	DD
3F7-4M	EE	CC	AA	JJ	FF	GG	EE	DD
3F7-5M	EE	CC	AB	JJ	FF	GG	EE	DD
3F7-6M	EE	CC	AA	JL	FF	GG	EE	DD
3F7-7M	EE	CC	AA	JJ	FF	GG	EE	DD
3F7-8M	EE	CC	AA	JJ	FF	GJ	EE	DD
3F7-9M	EE	CC	AA	JJ	FF	GG	EE	BD
3F7-10M	EE	CC	AB	JL	FF	GG	EI	DD
3F7-11M	EE	CC	AA	JJ	FF	GG	EE	DD
3F7-12M	EE	CC	AA	JJ	FF	GG	EE	DD
3F7-13M	EE	CC	AA	JJ	FF	GG	EE	DD
3F7-14M	EE	CC	AA	JJ	FF	GG	EE	BI
3F7-15M	EE	CC	AA	JJ	FF	GG	EE	DD
3F7-16M	EE	CC	AA	JJ	FF	GG	BI	DG
3F7-17M	EE	CC	AA	JJ	FF	GG	EE	DD
3F7-18M	EE	CC	AB	JJ	FF	GG	EE	DD
3F7-19M	EE	CC	AA	JJ	FF	GG	EE	DD
3F7-20M	EE	CC	AA	JJ	FF	GG	EE	DD
3F7-21M	EE	CC	AA	JL	FF	CG	EE	--
3F7-22M	EE	CC	AB	JJ	FF	GG	EE	DD
3F7-23M	EE	CC	AA	JL	FF	GG	EE	--
3F7-24M	EE	CC	AA	JJ	FF	GG	EE	DD
3F7-25M	EE	CC	AA	JJ	--	GG	EE	DD
3F7-26M	EE	CC	AA	JJ	--	GG	EI	DD
3F7-27M	EE	CC	AA	JJ	FF	GG	EE	DD
3F7-28M	EE	CC	AA	JJ	FF	GG	EE	DD
3F7-29M	EE	CC	AB	JJ	--	GG	EE	DG
3F7-30M	EE	CC	AA	JL	--	GG	EE	DD
3F7-31M	EE	CC	AA	JJ	FF	GG	EE	DD
3F7-32M	EE	CC	AA	JJ	FF	GG	EI	DD
3F7-33M	EE	CC	BB	JJ	FF	GG	EE	BD
3F7-34M	EE	CC	AA	JJ	--	GG	EE	DD
3F7-35M	EE	CC	AA	JL	--	GG	EE	DD
3F7-36M	EE	CC	AF	JJ	--	GG	EE	DD
VII-20-1989								
3F8-1M	EE	CC	AA	JJ	--	GG	EE	DI
VIII-09-1990								
3F9-2F	EE	CC	AF	JJ	FF	GG	EE	DD
3F9-3F	EE	CC	BB	JJ	FF	GG	EE	DD
3F9-1M	EE	CC	AB	JJ	FF	GG	EE	DD
Long Lake Pond, Alta.								
VI-22-1988								
3G2-2F	--	--	--	JJ	--	--	--	--
3G2-3F	EE	--	--	--	--	GG	--	--

Table A4.1, continued.

3G2-6F	--	--	--	J J	--	--	--	--
3G2-10F	--	--	--	J J	--	--	--	--
3G2-1M	--	--	--	J L	--	--	--	--
3G2-5M	--	--	--	J J	--	--	--	--
3G2-7M	--	--	--	J J	--	--	--	--
3G2-8M	EE	--	--	--	--	GG	--	--
3G2-9M	--	--	--	J J	--	--	--	--
VII-27-1988								
3G3-4F	--	--	--	J J	--	--	--	--
3G3-14F	EE	--	A A	J L	--	GG	EE	DD
3G3-15F	--	--	--	J J	--	--	--	--
3G3-16F	EE	--	B B	J J	--	GG	E I	DD
3G3-18F	EE	--	A B	J J	--	GG	EE	DD
3G3-20F	EE	--	A A	J J	--	EG	EE	DD
3G3-21F	EE	--	--	--	--	--	--	DD
3G3-22F	EE	--	A A	J J	--	GG	EE	DD
3G3-1M	EE	--	A A	J J	--	GG	E I	DD
3G3-2M	EE	--	A A	J J	--	EG	EE	DG
3G3-3M	--	--	--	--	--	--	--	DD
3G3-5M	EE	--	A A	J J	--	GG	EE	DD
3G3-6M	EE	--	--	J J	--	GG	EE	DD
3G3-7M	EE	--	A A	J J	--	C G	EE	DD
3G3-8M	EE	--	A A	J J	--	GG	EE	DD
3G3-9M	EE	--	A B	J L	--	GG	EE	DD
3G3-10M	--	--	--	J J	--	--	--	--
3G3-11M	EE	--	A A	J J	--	EG	EE	DD
3G3-12M	--	--	--	J J	--	--	--	--
3G3-13M	EE	--	A A	J J	--	GG	EE	DD
3G3-17M	EE	--	--	--	--	--	--	DD
3G3-19M	EE	--	--	--	--	--	--	DG
3G3-23M	EE	--	A A	J J	--	GG	EE	DG
3G3-24M	EE	--	A A	J J	--	GG	E I	DG
VIII-11-1988								
3G4-3F	EE	--	--	--	--	--	--	--
3G4-5F	EE	C C	A A	--	--	GG	EE	DD
3G4-8F	EE	--	--	--	--	GG	--	--
3G4-14F	EE	--	--	--	--	--	--	--
3G4-24F	--	--	--	--	--	--	EE	DD
3G4-6M	--	--	--	--	--	--	EE	B D
3G4-9M	--	C C	--	--	--	--	--	--
3G4-11M	EE	C C	A A	J J	--	GG	EE	DD
3G4-13M	--	C C	--	--	--	--	--	--
3G4-15M	--	--	--	--	--	--	E I	--
3G4-19M	--	C C	--	--	--	--	--	--
3G4-20M	--	--	--	--	--	--	EE	DD
3G4-21M	--	--	--	--	--	--	EE	DD
3G4-27M	EE	--	--	--	--	GG	--	--
3G4-28M	--	C C	--	--	--	--	--	--
3G4-7-	EE	C C	A A	J J	F F	GG	EE	DD
VII-07-1989								
3G5-1F	--	C C	A F	J J	F F	GG	EE	--
3G5-2F	EE	C C	A A	J L	F G	GG	EE	DD
3G5-9F	EE	--	--	J L	--	GG	EE	DD
3G5-11F	EE	C C	A A	J J	F F	GG	EE	DD
3G5-3M	--	C C	A A	J J	F F	GG	EE	DD
3G5-4M	--	C C	A A	G L	F F	GG	EE	D I
3G5-5M	EE	C C	A A	J J	F F	GG	EE	DD
3G5-6M	--	C C	A A	J J	F F	GG	EE	DG
3G5-7M	--	C C	A A	J J	F F	GG	EE	DD
3G5-8M	EE	--	B B	J J	--	GG	E I	DD
3G5-10M	EE	C C	A A	J J	F F	GG	EE	DD

Table A4.1, continued.

Hinton, Alta.								
VII-11-1989								
3H2-7F	EE	CC	AA	JJ	FF	GG	EE	DD
3H2-8F	EE	CC	AA	JJ	FF	GG	BI	BD
3H2-9F	EE	CC	AA	JJ	FF	CG	EE	DD
3H2-10F	EE	CD	AA	JJ	FF	CG	EE	DD
3H2-11F	EE	CC	AA	JJ	FF	GG	EE	DD
3H2-1M	EE	CC	AA	JJ	FF	GG	EE	DD
3H2-2M	EE	CC	AB	JJ	FG	GG	EE	BD
3H2-3M	EE	CC	AA	JJ	FF	GG	EI	DD
3H2-5M	EE	CC	AA	JJ	FF	GG	EE	BD
VII-31-1989								
3H3-3F	EE	CC	AF	JJ	FF	GG	EE	DG
3H3-4F	EE	CC	AB	JJ	FF	GG	EE	DD
3H3-6F	EE	CC	AA	JJ	FF	GG	EE	DD
3H3-10F	EE	CC	AB	JJ	FF	GG	EE	DD
3H3-11F	EE	CC	AF	JJ	FF	GG	EE	--
3H3-16F	EE	CC	AF	JL	FF	GG	EE	DD
3H3-17F	EE	CC	AA	JJ	FF	GG	EE	DG
3H3-18F	EE	CC	AA	JJ	FF	GG	EE	--
3H3-1M	EE	CC	AF	JL	FF	GG	EE	DD
3H3-2M	EE	CC	AB	JJ	FF	GG	EE	DD
3H3-5M	EE	CC	AA	JL	FF	GG	EI	BD
3H3-9M	EE	CC	AA	JL	FF	GG	EE	DD
3H3-12M	EE	CC	AA	JJ	FF	GG	EI	DD
3H3-13M	EE	CC	AA	JJ	FF	GG	EE	DD
3H3-14M	EE	CC	AB	JJ	FF	GG	EE	DD
3H3-19M	EE	CC	AA	JL	FF	GG	EE	DD
Lynch Lakes, Alta.								
VII-01-1989								
3I2-9F	EE	CC	AA	JJ	FF	GG	EE	DD
3I2-10F	EE	CC	AA	JJ	FF	GG	EE	DD
3I2-23M	EE	CC	BF	JJ	FF	GG	EE	DD
3I2-24M	EE	CC	AA	JJ	FF	GG	EE	DD
3I2-25M	EE	CC	BF	JJ	FF	EG	EE	DD
3I2-26M	EE	CC	AA	JJ	FF	GG	EE	DD
3I2-27M	EE	CC	AA	JJ	FF	GG	EE	DD
VII-20-1989								
3I3-15F	EE	CC	AB	JJ	FF	EG	EE	DD
3I3-17F	EE	CC	AA	JL	FF	EG	EI	DD
Wauconda, Washington								
VII-18-1989								
3J2-12F	EE	CC	AA	JJ	FF	GG	EI	DD
3J2-15F	EE	CC	AA	LL	FF	GG	EE	DD
3J2-28F	EE	CC	FF	LL	FF	GG	EE	DD
3J2-18M	EE	CC	AA	JL	--	GG	EE	DD
3J2-19M	EE	CC	AA	JJ	FF	GG	EE	DD
3J2-22M	EE	CC	AA	JL	FF	GG	EI	DD
3J2-24M	EE	CC	AA	JL	FF	GG	EE	DI
3J2-27M	EE	CC	AA	JL	FF	GG	EI	DD
3J2-29M	EE	CC	AA	JL	FF	GG	EI	DD

P. fossifrons/stygicus hybrid

Wauconda, Washington								
VII-18-1989								
4A2-20F	EG	CC	FF	KL	FF	FF	EE	II

Table A4.1, continued.

P. lecontei								
Lynch Lakes, Alta.								
VII-01-1989								
5A2-3F	FF	CC	AA	BB	GG	HH	CC	FF
5A2-4F	FF	CC	CF	BB	GG	HH	CC	FF
5A2-7F	FF	CC	FF	BB	GG	HH	CC	FF
5A2-1M	FF	CC	--	BB	GG	HH	CC	FF
5A2-5M	FF	CC	--	AA	--	HH	CC	FF
5A2-2-	FF	CD	--	BB	GH	HH	CC	FF
5A2-6-	FF	CC	FF	BB	GG	HH	CC	FF
VII-20-1989								
5A3-13-	FF	CC	CF	BB	GG	HH	CE	FF
5A3-20-	FF	CC	FF	BB	GG	HH	CC	FF
Curlew Pond, Alta.								
VII-11-1988								
5C2-6F	FF	CC	AA	BB	GG	HH	CE	FF
George Lake, Alta.								
VII-19-1988								
5D2-12F	FF	CC	AA	BB	GG	HH	CC	FF
Tern Pond, Alta.								
VI-29-1989								
5E2-1M	FF	CC	FF	BB	GG	HH	CC	FF
Rock Pond, Alta.								
VI-29-1989								
5F2-2F	FF	CC	AC	BB	GG	HH	CC	FF
5F2-1M	FF	CC	CC	BB	GG	BH	CC	--
5F2-3M	FF	CC	CC	BB	GG	HH	CC	FF
Bennett Billabong, Alta.								
VI-30-1989								
5G2-1F	FF	CC	AA	BB	GG	HH	CE	FF
Pecten Pond, Alta.								
VI-30-1989								
5H2-1F	FF	CC	AC	BB	GG	HH	CC	FF
P. longicornis								
Edmonton, Alta.								
VIII-08-1990								
11A2-3F	FF	CC	CC	GJ	EE	AE	CC	FI
11A2-4F	FF	CC	CC	EG	EE	AA	CC	FF
11A2-6F	FF	CC	CC	EG	EE	AA	AA	FF
11A2-7F	FF	CC	CC	GG	EE	AD	AC	FF
11A2-9F	FF	CC	CC	EE	EE	AA	CC	FF
11A2-1M	FF	CC	CE	EG	EE	DD	CE	FF
11A2-2M	FF	CC	CC	EJ	EE	AD	CC	FF
11A2-5M	FF	CC	CC	GG	EE	AD	AC	FF
11A2-8M	FF	CC	CE	EG	EE	AA	CC	II
P. foveocollis								
Hinton, Alta.								
VII-11-1989								
8A2-6F	EE	DD	FF	BB	GG	CC	BB	AA
Long Lake Pond, Alta.								
VIII-11-1988								
8B2-31F	EE	CC	--	BB	GG	CD	BB	AA

Table A4.1, continued.

P. septentrionis								
Hinton, Alta.								
VII-11-1989								
7A2-4M	EE	CC	AE	BB	FF	HH	--	--
VII-31-1989								
7A3-7F	EE	CC	AF	BB	FF	HH	DD	--
7A3-8M	EE	CC	FF	BB	FF	HH	DD	CC
7A3-15M	EE	CC	FF	BB	FF	HH	DD	CC
7A3-21M	EE	CC	FF	BB	FF	HH	DG	CC
7A3-22M	EE	CC	EE	BB	FF	HH	GG	CC
P. atrorufus								
Finland								
1989								
6A2-1F	FF	CC	CC	CG	FF	BK	IK	GG
6A2-4F	FF	CC	CC	CE	FF	BB	GI	GG
6A2-8F	FF	CC	--	CE	FF	BI	IK	GG
6A2-11F	FF	CC	--	CC	DD	HH	BE	DD
6A2-3M	FF	CC	--	GG	FF	BB	II	GG
6A2-6M	FF	CC	--	CE	FF	BB	GI	FG
6A2-7M	FF	CC	--	GG	FF	BB	GG	GG
6A2-9M	FF	CC	--	EG	FF	BB	IK	GG
6A2-10M	FF	CC	CE	CE	FF	BB	GI	GG
Diplous aterrimus								
Rocky Mtn. House, Alta.								
VI-14-1989								
9A2-3F	CC	AA	--	DH	--	KK	FH	HJ
9A2-6F	CD	AA	DG	HH	--	KK	HH	EJ
9A2-14F	CD	AA	--	HH	--	KK	HH	JJ
9A2-17F	CD	--	DD	DH	--	KK	HH	--
9A2-18F	CD	--	BD	DH	--	KL	HH	--
9A2-23F	BB	AA	BD	HH	--	KK	HH	--
9A2-26F	CD	AA	DD	HH	--	KK	FH	EH
9A2-1M	CC	AA	DD	HH	--	KK	HH	EH
9A2-5M	DD	AA	DD	DH	--	KK	HH	EJ
9A2-7M	CC	AA	--	HH	--	KK	FH	HJ
9A2-8M	CC	AA	--	HH	--	KL	--	EJ
9A2-9M	DD	AA	--	HH	--	KK	HH	HH
9A2-13M	CC	AA	BB	DH	BB	KK	HH	EH
9A2-16M	--	AA	--	HH	BB	KK	BH	EJ
9A2-19M	BC	--	DD	HH	--	KK	HH	--
9A2-20M	DD	AA	BD	HH	--	KK	HH	--
9A2-21M	CC	AA	BD	HH	--	KK	FH	--
9A2-22M	BC	AA	DD	HH	--	KM	HH	--
9A2-24M	BC	AA	BG	HH	--	KK	HH	EH
9A2-25M	CC	AA	BG	HH	--	KK	HH	EE
9A2-27M	CD	AA	DD	HH	--	KK	HH	EJ
9A2-28M	CD	AA	DD	HH	--	KK	HH	EE
9A2-29M	CC	AA	DD	HH	--	KK	HH	EJ
9A2-30M	DD	--	BB	HH	--	KK	HJ	--
9A2-2-	BD	AA	BD	HH	--	KK	HH	EE
9A2-4-	DD	AA	BB	DD	--	KK	FH	EJ
9A2-10-	--	--	BB	DH	--	KM	FH	EE
9A2-11-	--	--	DD	HH	--	KK	HH	EJ
VII-03-1989								
9A3-1F	CD	AA	DG	HH	BB	IK	HH	--
9A3-3F	BC	AA	DD	HH	BB	KK	FH	HH
9A3-5F	--	AA	DD	HH	BB	KK	HH	EH
9A3-6F	--	AA	DD	HH	BB	KK	HH	EJ
9A3-8F	--	AA	--	HH	BB	KK	FF	EJ

Table A4.1, continued.

9A3-9F	--	AA	DG	HH	BB	KK	FH	EH
9A3-11F	--	AA	DD	HH	BB	KK	FH	EH
9A3-13F	--	AA	--	HH	BB	KK	HH	EJ
9A3-14F	--	AA	DD	HH	BB	KK	HH	EE
9A3-15F	--	AA	DD	HH	BB	KK	HH	EJ
9A3-2M	CC	AA	DG	HH	BB	KM	FF	HJ
9A3-10M	--	AA	--	DH	BB	KK	HH	EH
9A3-12M	--	AA	--	HH	BB	KK	HH	EH
9A3-16M	CC	AA	DG	HH	BB	KL	FF	JJ
9A3-17M	BB	AA	DG	HH	BB	KK	HH	JJ
9A3-18M	CC	AA	BB	HH	BB	KK	HH	EH
9A3-19M	--	AA	DD	HH	BB	KK	FH	EJ
9A3-20M	CD	AA	DD	HH	BB	KK	HH	HJ
9A3-21M	BB	AA	DD	HH	BC	KM	FH	EH
9A3-22M	BC	AA	DD	HH	BB	KK	FH	HH
Waterton River, Alta. VI-27-1989								
9B2-1F	--	AA	DD	HH	BB	--	--	--
9B2-5F	CD	AA	DD	HH	BB	KM	FH	HJ
9B2-6F	BC	AA	DG	HH	BB	MM	HH	JJ
9B2-2M	CC	AA	DD	DH	BB	KM	FH	EH
9B2-3M	CC	AA	DD	HH	BB	KM	FH	EH
9B2-4M	CC	AA	DD	HH	BB	KM	HH	EE
Oldman River, Alta. VI-27-1989								
9C2-1M	CD	AA	DD	DH	BB	KK	HH	EH
Diplous californicus								
Similkameen River, B.C. VII-13-1989								
10A2-1F	--	AA	CG	FF	BB	NN	HJ	CC
10A2-2F	BB	AA	CG	FH	BB	NN	HH	CC
10A2-3F	--	AA	CG	FF	AB	NN	HH	CF
10A2-4F	--	AA	CC	FF	BB	NN	HH	CC
10A2-5F	BB	AA	CC	FF	BB	NN	HH	CC
10A2-6F	--	AA	CC	FH	BB	NN	HJ	CC
10A2-7F	BB	AA	CC	FF	BB	NN	HJ	BC
10A2-8F	BB	AA	CC	FH	BB	NN	HH	CC
10A2-9F	--	AA	GG	FF	AB	NN	HJ	CC
10A2-10F	--	AA	GG	FH	BB	NN	HJ	CF
10A2-11F	BB	AA	--	FH	BB	NN	HH	CC
10A2-12F	--	AA	--	FF	BB	NN	HH	BB
10A2-13F	BB	AA	CC	FF	BB	NN	HH	CF
10A2-14F	BB	AA	GG	FF	BB	NN	HH	CF
10A2-15F	--	AA	GG	FF	BB	NN	HH	FF
10A2-16M	BB	AA	GG	FF	BB	NN	HH	CF
10A2-17M	BB	AA	GG	FF	BB	NN	HJ	CC
10A2-18M	BB	AA	GG	HH	BB	NN	HH	CF
10A2-19M	BB	AA	CG	FF	BB	NN	HH	CC
10A2-20M	AB	AA	--	FH	BB	NN	JJ	CC
10A2-21M	BB	AA	CG	FH	BB	NN	HJ	BC
10A2-22M	BB	AA	--	FF	BB	NN	HJ	CF
10A2-23M	BB	AA	CC	FH	BB	NN	HJ	CC
10A2-25M	BB	AA	CG	FF	AB	KN	HJ	CF
10A2-26M	BB	AA	GG	FF	BB	NN	HJ	CF
10A2-27M	BB	AA	--	FF	BB	NN	HJ	CC
10A2-29M	--	AA	--	FF	BB	NN	HJ	CF
10A2-30M	BB	AA	--	FH	BB	NN	HH	CC
10A2-35M	BB	AA	GG	HH	BB	NN	HJ	CC
10A2-38M	BB	AA	--	FH	BB	NN	HH	CC
10A2-39M	BB	AA	--	FF	BB	NN	HJ	CF
10A2-40M	--	AA	CC	FF	BB	NN	HH	CC
10A2-41M	BB	AA	GG	FH	AB	KN	HJ	CC

APPENDIX #5. MORPHOLOGICAL DATA

Table A5.1: measurements (in micrometer units) of quantitative morphological characters, and character states of qualitative morphological characters. Pronotum W = width; pronotum L = length; antenna 9L, 10L, 11L, and 9W = lengths of antennal segments nine, 10, and 11, and width of segment nine, respectively; M = elytral microsculpture shape, from 1 (isodiametric) to 5 (transverse); G = shape of median lobe of male genitalia, from 1 (spoon shaped) to 5 (fish-hook shaped); W = state of development of hindwing (0 = brachypterous; 1 = macropterous); wing character I = presence of secondary sclerotized island (0 = absent; 1 = present, normal; a = present, attached to primary sclerotized island; d = present, deformed; t = present, tiny); R1-vein character A = length of wide sclerotized area on R_1 vein; R1-vein character B = total length of R_1 vein; wedge character C = width of wedge cell (m = wedge cell missing); wedge character D = length of cubital vein, between the two cubito-anal cross-veins. Blanks indicate missing data.

SAMPLE, CODE, & SEX	PRO-NOTUM		ANTENNA				M	G	W	LEFT WING					RIGHT WING				
										I	R1-VEIN		WEDGE		I	R1-VEIN		WEDGE	
	W	L	9L	10L	11L	9W					A	B	C	D		A	B	C	D
P. fossifrons (inland)																			
Rock Pond, Alta.																			
VI-29-1989																			
1A2-2F	74	57	20	19	28	10	1		1	1	42	49	7.5	53	1	43	52	8.5	51
1A2-3F	80	57	20	20	28	10	1		1	1	44	53	8	57	1	43			53
1A2-5F	75	59	21	21	28	10	1		1						1	43	52	9	55
1A2-6F	75	58	21	21	28	10	2		1	1	46	53	8	50	1	46	54	7	52
1A2-7F	81	57	20	20	28	10	1		1						1	43	49	8.5	55
1A2-8F	78	59	20	21	28	10	1		1									8	57
1A2-9F	65	49	19	19	26	9	1		1	1	36	44	6.5	47	1	36	43	6.5	45
1A2-10F	72	53	21	20	27	10	1		1	1	39	45	8	54	1	39	46	8	54
1A2-11F	70	53	20	20	26	10	1		1	1	39	47	8.5	52	1	39	47	8.5	51
1A2-12F	71	56	21	21	27	10	1		1	1	47	55	6	54	1	46	54	6.5	54
1A2-13F	72	54	20	20	27	10	1		1						1	42	50	7	52
1A2-14F	77	57	20	21	28	10	1		1						1	43	53	10	57
1A2-15F	72	56	22	21	28	10	1		1	1	47	54	8.5	55	1	43	53	8.5	56
1A2-16F	70	53	20	20	28	9	1		1	1	44	50	8.5	52	1	46	50	8	52
1A2-17F	81	58	20	21	29	10	1		1	1	45	50	9	58	1	46	51	9	56
1A2-18F	75	56	21	21	28	10	1		1	1	43	50	6.5	55	1	45	53	7	56
1A2-19F	73	55	21	21	28	10	1		1	1	42	49	7	52	1	41	49	6.5	53
1A2-20F	68	52	19	19	25	10	1		1	1	44	50	9.5	53	1	42	50	9	54
1A2-21F	80	60	20	22	30	10	1		1	1	50	57	9	59	1	49	55	9	60
1A2-22F	72	55	21	20	28	9	1		1	1	44	49	6	54	1	45	49	7	52
1A2-23F	73	57					1		1	1	47	54	9	56	1	47	54	8.5	57
1A2-24F	72	53	21	19	28	9	1		1	1	44	51	9	54	1	45	51	9	54
1A2-25F	78	56	21	21	28	10	1		1	1	47	54	9	56	1	47	54	8.5	57
1A2-26F	72	52	20	20	27	10	1		1	1	47	53	7.5	53	1	46	52	7.5	52
1A2-44F	72	50	20	19	27	10	1		1										
1A2-45F	70	51					1		1										
1A2-46F	70	53	20	20	28	10	1		1										
1A2-47F	73	53	21	20	29	10	1		1										
1A2-48F	65	52	20	20	27	9	1		1										
1A2-P54F	75	55					3												
1A2-P62F	75	56	20	20	27	10	1												
1A2-P68F	68	52	20	20	26	10	1												
1A2-P69F	69	57	20	21	29	10	1												
1A2-1M	68	50	20	20	26	10	1		1	1	38	46	8	48	1	34	47	8	49
1A2-4M	74	57	21	20	29	10	2		1	1	44	51	9	56	1	45	52		54
1A2-27M	72	55	22	21	28	10	1		1	1	46	52				43			
1A2-28M	63	49	19	18	24	9	1		1	1	37	41	6.5	49	1	38	42	6.5	47
1A2-29M	69	55	23	22	30	10	1		1		41	49	8.5	55	1	40	47	m	54
1A2-30M	73	57	22	21	29	10	1		1	1			8	49	1	44	52	7.5	51
1A2-31M	71	54	21	20	28	10	1		1	1	45	53	7	53	1	43	52	7.5	53

Table A5.1, continued.

1A2-32M	72	55	20	20	28	10	1		1	1	42	49	7	51	1	41	48	7.5	52
1A2-33M	70	51	21	20	28	10	1		1	1	44	50	6.5	51	1	44	49	5.5	52
1A2-34M	74	57	21	22	27	10	2		1						1	46	52	8	54
1A2-35M	70	54	22	21	28	10	1		1	1	42	48	7.5	49	1	41	49	7	51
1A2-36M	74	53	23	22	31	10	1		1	1	43	50	7.5	53	1	43	49	7.5	51
1A2-37M	69	53	21	21	28	10	1		1	1			8.5	45	1	43	51	7.5	46
1A2-38M	73	56	21	21	30	10	1		1										
1A2-39M	69	52	21	21	28	9	1		1										
1A2-40M	70	52	21	21	28	10	1		1	1	42	49	m	47	1	42	48	7	47
1A2-41M	74	55	23	22	30	10	1		1	1	43	50	8	57	1	43	52	8	58
1A2-42M	66	52	20	19	25	10	1		1										
1A2-43M	69	51	21	20	27	10	1		1	1	38	45	8.5	48	1	40	48	8	48
1A2-49M	66	54	20	21	27	10	1		1										
1A2-50M	66	51	21	21	28	10	1		1										
1A2-51M	73	53	21	21	28	10	1		1										
1A2-52M	62	51	19	20	27	10	1		1										
1A2-53M	68	53	22	22	28	10	1		1										
1A2-P56M	71	53	21	22	28	10	1												
1A2-P57M	68	51	21	21	28	10	1												
1A2-P58M	69	52	21	21	28	10	1												
1A2-P59M	68	52	22	22	29	10	1												
1A2-P60M	69	55	21	21	29	10	1												
1A2-P61M	69	54	20	20	26	10	3												
1A2-P63M	69	53	22	21	27	10	1												
1A2-P64M	72	55	21	21	29	10	1												
1A2-P65M	68	49	22	22	29	10	1												
1A2-P66M	71	52	21	22	29	10	1												
1A2-P67M	68	52	22	21	29	9	2												
1A2-P70M	68	50	21	20	27	9	2												
1A2-P71M	69	53	22	22	30	10	2												
Tern Pond, Alta.																			
VI-29-1989																			
1B2-7F	66	50	19	19	27	10	1		1	1	41	47	7.5	44	1	41	47	m	45
1B2-8F	73	53	21	21	28	10	1		1	1	46	52	8.5	55	1	48	52	8.5	55
1B2-10F	74	55	21	21	28	10	1		1	1	45	55	8.5	54	1	45	53	7.5	54
1B2-11F	71	55	21	20	29	10	1		1	1	44	52	8.5	55	1	42	50	9	55
1B2-12F	72	56	22	21	27	10	1		1	1	45	52	7	56	1	45	52	7.5	55
1B2-13F	70	55	20	21	28	10	1		1	1	46	53	8.5	56	1	44	55	8.5	57
1B2-14F	69	53	21	20	27	9	1		1	1	45	52	8.5	56	1	44	50	9	56
1B2-15F	70	52	20	20	27	10	1		1	1	42	49	m	51	1	42	48	7.5	53
1B2-16F	65	49	19	18	27	9	1		1	1	42	50	8	45	1	42	50	7.5	45
1B2-17F	72	54	20	21	29	10	1		1	t	42	48	6	50	0	39	48	9	54
1B2-18F	75	58	21	22	29	10	1		1	1	47	55	1	57	0	47	56	9.5	57
1B2-19F	70	48	20	20	26	9	1		1	1	40	45	7.5	53	1	40	46	8	51
1B2-1M	70	52	22	21	28	10	1		1	1			7	51		42	50		
1B2-2M	65	50	21	21	28	10	1		1	1	40	47	7.5	49	1	39	46	6.5	49
1B2-3M	64	50	20	20	26	9	1		1	1	38	45	6	48	1	39	45	7.5	47
1B2-4M	68	52	21	21	29	10	1		1	1	41	49	8.5	47	1	39	47	m	46
1B2-5M	73	54	23	23	29	10	1		1	1	44	51	6.5	54	1	42	50		53
1B2-6M	67	52	22	21	29	10	1		1	1	46	52	9	53	1	46	52	8.5	52
1B2-9M	72	56	21	20	29	10	1		1	1	41	50			1	40	48	8.5	53
1B2-P20M	69	51	21	20	28	10	1												
Lynch Lakes, Alta.																			
VII-01-1989																			
1C3-1F	72	56	21	21	30	10	1		1	1	43	51	8	54	1	44	54	8.5	54
1C3-2F	70	56	21	21	27	10	1		1	1	45	53	9.5	54	1	46	54	8.5	54
1C3-3F	76	57	21	21	29	10	1		1	1	43	50	8	56	1	44	52	7.5	55
1C3-4F	72	53	21	23	28	10	1		1	1	45	54	7.5	56	1	45	57	7.5	56
1C3-5F	69	54	20	20	27	9	1		1	t	45	50	8.5	50	1	44	52	7.5	49
1C3-6F	75	57	21	21	30	10	1		1	a	42	49	8	58	a	43	50	8.5	58
1C3-7F	75	56					1		1	1	47	57	8	59	1	46	54	8	58
1C3-8F	76	58	22	21	29	10	1		1	1	43		9.5	58	1	45	50		58
1C3-11F	79	57	21	21	29	10	1		1	1	49	55	8.5	56	1	47	55	8	55

Table A5.1, continued.

1C3-12F	69	53	20	20	26		1		1	1	43	50	9	52	1	43	52	m	51
1C3-13M	74	57	23	23	31	10	1		1	1	45	52	9	52	1	45	52	8.5	54
1C3-14M	71	53	22	21	28	10	1		1	1	41	48	8	54	1	41	49	9	54
1C3-15M	70	53	21	21	27	10	1		1	1	36	42	7.5	49	1	37	45	7.5	49
1C3-16M	68	51	21	21	28	10	1		1	1	39	46	8	49	1	39	46	8	49
1C3-17M	70	51	22	21	28	10	1		1	1	41	49	8.5	50	1	41	50	8	49
1C3-18M	75	57	22	22	30	10	1		1	1	41	48	9	57	1	40	47	7.5	57
1C3-19M	72	53	23	23	30	10	1		1	1	42	51	7	56	1	43	53	7.5	54
1C3-20M	69	52	22	21	28	10	1		1	1	39	48	7.5	48	1	40	48	7.5	48
1C3-21M	75	53	21	23	28	10	1		1	1	42	51	8	53	1	42	52	8	54
1C3-22M	68	51	21	21	28	10	1		1	1	42	50	7.5	51	1	41	49	8	52
VII-20-1989																			
1C4-1F	68	52	21	20	27	10	1		1	1	41	48	9	55	1	39	48	8	56
1C4-2F	75	55	22	21	28	10	1		1	1			8.5	52	1	45	52	7.5	59
1C4-5F	73	55	21	20	26	10	1		1	1	43	50	9	54	1	43	51	8.5	
1C4-6F	81	59	21	21	28	10	1		1	1	43	51	8	54	1	44	52	8.5	57
1C4-7F	72	58	21	20	28	10	1		1	1	41	48	8	54	1	41	48	8	55
1C4-9F	73	54	21	21	27		1		1	1	44	52	8.5	53	1	43	51	7	51
1C4-12F	73	55			26		1		1		44	52	8	54	a	44	50	8.5	52
1C4-P21F	78	56	20	20	28	10	1												
1C4-3M	76	56	23	23	29	10	1		1	1	47	53	8	53	1	45	52	8.5	53
1C4-4M	74	55	23	23	30	10	1		1	1	43	50	8	54	1	44	52	8	54
1C4-8M	70	53	22	21	29	10	1		1	1	41	48	6.5	53	1	42	49	6.5	51
1C4-10M	69	53	21	21	30	10	1		1	1	40	49	7.5	52	1	39	47	8.5	52
1C4-11M	73	52	23	22	30	10	2		1	1	42	49	7.5	53	1	43	51	7.5	54
1C4-14M	68	49	20	20	27	10	1		1	1	39	45	7.5	47	1	38	45	8	48
1C4-16M	72	55	20	21	26	10	1		1	1	39	44	8.5	51	1	37	44	8	50
1C4-18M	72	54	23	23	29	10	1		1	1	42	51	8.5	52	1	43	52	9	50
1C4-19M	74	57	23	23	30	11	1		1	1	43	50	8	57	1	42	52	7.5	57
Bull Pond #1, Alta.																			
VII-09-1987																			
1E1-P85M	69	54	22	22	28	10	2	1	1	1	41	49	7.5	53	1	42	51	8	54
1E1-P86M	68	50	21	21	28	10	2	2	1	1	41	51	8	49	1	40	50	m	50
Pothole Pond, Alta.																			
VII-07-1987																			
1F1-P63F	70	53	22	22	32	10	1												
1F1-P64F	69	50	20	20	28	10	1												
1F1-P65F	68	50	21	21	29	10	1												
1F1-P92M	66	52	22	22	30	10	2												
1F1-P93M	69	51	22	22	29	10	1												
1F1-P94M	70	52	22	23	31	10	2												
1F1-P95M	67	51	22	22	30	10	2												
1F1-P96M	66	52	22	22	29	10	2												
1F1-P97M	67	49	23	22	29	10	1												
1F1-P98M	70	53	22	23	29	10	1												
VI-07-1989																			
1F2-1F	78	56	22	22	29	10	1		1	1	47	54	9	59	1	45	56	9	59
Kavanaugh's Pond, Alta.																			
VII-18-1970																			
1G1-P-1F	68	51	20	21	27	10	1												
1G1-P-2F	74	55	20	21	28	10	1												
1G1-P-3F	76	53	22	21	30	10	2												
1G1-P-4F	76	57	22	22	28	10	1												
1G1-P-5F	71		21	21	29	10	1												
1G1-P-6F	76	55	21	21	30	10	2												
4B1-P-2F	73	54	21	21	29	9	3		1	1	43	53	8.5	56	1	44	52	8.5	56
1G1-P-8M	69	53	22	21	26	10	2	1											
1G1-P-9M	72	53	22	23	31	10	1	1											
1G1-P10M	69	50	22	23	29	10	1	1											
1G1-P11M	69	52	22	22	28	10	1	1											
1G1-P12M	65	50	20	21	28	10	2	1											
1G1-P13M	73	55	20	20	29	10	2	1											
1G1-P14M	73	53	23	23	28	10	1	1											

Table A5.1, continued.

1G1-P15M	70	50	23			10	2	2												
1G1-P16M	67	52	23	22	29	10	2	1												
1G1-P17M	72	53	22	22	28	10	1	2												
1G1-P18M	75	54	22	23	30	10	2	1												
1G1-P19M	69	49	21	21	28	10	1	1												
1G1-P20M	72	53	22	22	31	10	2	1												
1G1-P21M	72	51	21	21	29	10	1	2												
1G1-P22M	72	53	22	22	29	10	2	1												
1G1-P23M	66	48	21	22	29	10	1	1												
1G1-P24M	72	52	22	23	30	10	1	1												
Arco, Idaho																				
VII-30-1960																				
1H1-P47F	76	57	20	20	26	11	1		0											
1H1-P48F	71	53	19	19	24	10	1		0											
1H1-P49F	75	55	19	19	27	10	1		0											
1H1-P50F	74	56	19	20	27	10	1		0											
1H1-P35M	65	49	20	21	18	10	1	1	0											
1H1-P36M	69	49	20	20	27	11	1	1	0											
1H1-P37M	64	46	19	19	26	10	1	1	0											
1H1-P38M	64	49	19	19	25	10	1	2	0											
1H1-P39M	71	54	22	21	16	10	1	1	1	a	43	56	8.5	52	0	43	51	9	52	
1H1-P40M	72	54	21	20	27	10	1	2	0											
1H1-P41M	71	53	21	20	26	10	1	1	0											
1H1-P42M	72	52	20	20	26	10	1	1	0											
1H1-P45M	68	51	20	20	25	10	1	2	0											
Beaverhead Co., Montana																				
IX-04-1966																				
1I1-P51F	73	56	22	21	30	11	1		0											
1I1-P52F	71	54	20	20	27	10	1		0											
1I1-P53F	70	52	19	19	26	11	1		1	1	43	50	8	49	1	42	50	9.5	48	
1I1-P54F	74	58	20	20	26	11	1		0											
1I1-P43M	74	55	22	22	29	10	1	1	0											
1I1-P44M	69	52					1	2	1	1	42	52	8.5	50	1	41	54	7.5	51	
1I1-P46M	66	48	20	20	26	10	1		0											
Cypress Hills, Alta.																				
VII-03-1957																				
1J1-P97M	71	53	24	23	31	11	1	2	1	1	41	55	7.5	52	1	44	55	7.5	52	
1J1-P98M	66	50	20	19	27	10	1	2	1	1	44	55	7	45	1	42	51	9.5	46	
Ball's Pond, Alta.																				
VII-26-1956																				
1K1-P31F	75	57	20	21	29	9	1		1											
1K1-P32F	69	53	21	21	27	10	2		1											
1K1-P33F	61	47	18	19	24	9	1		1											
1K1-P34F	73	53	21	21	27	10	1		1											
1K1-P35F	71	52	19	19	26	9	1		1											
1K1-P36F	74	57	21	21	30	10	1		1											
1K1-P37F	74	57	21	21	28	10	1		1											
1K1-P38F	73	53	20	20	27	10	1		1											
1K1-P39F	75	56	20	21	29	10	1		1											
1K1-P40F	76	55	19	19	26	10	2		1											
1K1P101F	69	50	21	22	27	9	3		1											
1K1-P29M	70	52	22	22	29	11	1	2	1											
1K1-P30M	70	54	21	21	28	11	2	2	1											
1K1-P31M	64	49	19	19	26	10	1	3	1											
1K1-P32M	70	52	21	21	28	10	1	2	1											
1K1-P33M	68	50	20	20	26	10	1	3	1											
1K1-P34M	65	51	20	21	28	10	1	3	1											
1K1-P35M	69	51	21	21	27	10	1	2	1											
1K1-P36M	69	53	20	20	27	10	1	3	1											
1K1-P37M	68	50	22	21	29	10	1	2	1											
1K1-P38M	64	47	19	20	26	9	1	2	1											
1K1-P39M	65	51	20	21	28	10	1	2	1											
1K1-P40M	63	48	19	19	27	10	1	2	1											

Table A5.1, continued.

1K1-P41M	72	53	21	20	28	10	1	2	1											
1K1-P42M	69	52	23	21	29	11	1	2	1											
1K1-P43M	67	52	20	20	26	10	1	3	1											
1K1-P44M	73	57	23	23	32	10	2	3	1											
1K1-P45M	68	51	21	21	28	10	1	2	1											
1K1-P46M	63	48	20	20	26	10	1	3	1											
1K1-P47M	65	51	17	17	23	10	1	2	1											
1K1-P48M	67	50	20	20	26	10	1	3	1											
1K1-P49M	70	50	20	21	27	10	1	2	1											
1K1-P50M	70	53	20	20	28	10	1	2	1											
1K1-P51M	73	55	22	21	30	11	1	2	1											
1K1-P52M	69	53	22	20	27	11	1	2	1											
1K1-P53M	68	52	21	21	27	10	1	2	1											
Curlew Pond, Alta.																				
VII-11-1988																				
1L2-1F	74	53	20	22	33	10	1		1	a	45	55		60	1	45	54	8.5	51	
1L2-3F	72	55	22	22	28	10	1		1	1	49	58	9	54	1	46	55	m	54	
1L2-5F	76	57	22	21	29	10	1		1	1	42	52	7.5	50	1	44	55	8	49	
1L2-2M	65	45	22	21	28	10	1		1		41	49	7	46	1	40	47	7.5	47	
1L2-4M	70	57	24	24	30	10	1		1	1			7.5	51	1	43	53	7.5	52	
Del Bonita, Alta.																				
VI-10-1955																				
4C1-P59F	73	57	22	21	30	11	2		1	a	44	54	10	54	a	45	52	m	54	
P. fossifrons (coastal)																				
Manning, B. C.																				
VII-13-1989																				
2A2-1F	57	44	17	16	24	9	1		1	0	41	48	9	48	0	41	45	9	45	
Deep Pond, B.C.																				
VI-13-1989																				
2B2-1M	67	50	19	19	26	10	3		0											
Train Pond, B.C.																				
VI-13-1989																				
2C2-1M	71	55	21	22	28	10	1		0											
Creston, B.C.																				
V-19-1958																				
2D1-P83M	61	47	17	17	24	10	1	1	1	0	39	45	9	45	0	39	45	7.5	45	
Wauconda, Washington																				
VII-18-1989																				
1D2-1F	69	52	17	19	26	9	1		0											
1D2-2F	71	54	17	18	25	9	2		0											
1D2-4F	70	55	18	19	24	9	2		0											
1D2-5F	73	57	19	20	27	10	1		0											
1D2-7F	70	54	19	19	24	10	1		0											
1D2-9F	70	54	20	19	25	10	1		0											
1D2-11F	70	54	18	18	24	9	1		0											
1D2-14F	71	53	18	19	26	10	1		0											
1D2-16F	73	56	18	18	26	10	2		0											
1D2-23F	75	55	19	19	26	10	1		0											
1D2-25F	67	54	17	18	25	10	1		0											
1D2-P-3M	69	53	19	19	26	10	2	2												
1D2-6M	71	54	19	20	26	9	1		0											
1D2-8M	64	51	20	20	25	10	1		0											
1D2-10M	70	55	19	20	25	9	1		0											
1D2-13M	70	52	18	19	26	10	1		0											
1D2-17M	69	52	19	20	27	10	1		0											
1D2-21M	68	55	19	20	27	10	1		0											
1D2-26M	71	56	20	20	27	10	1		0											
1D2-P30M	64	47	17	18	24	10	3	2	0											
St. Mary's Lake, B.C.																				
VIII-27-1955																				
2F1-P49F	64	47	17	17	24	10	1													
2F1-P50F	64	50	17	17	25	10	1													
2F1-P51F	63	47	18	18		9	1													

Table A5.1, continued.

2F1-P52F	62	45	17	17	24	10	1	
2F1-P53F	65	45	17	17	25	9	1	
2F1-P54F	62	47	18	17	26	10	1	
2F1-P62M	62	46					1	1
2F1-P79M	54	42	17	17	24	9	1	1
Crowsnest Lake, Alta.								
various dates								
2G1-P47F	60	45	15	16	22	9	1	
2G1-P48F	64	48	16	16	22	10	1	
2G1-P64M	62	46	17	17	25	10	1	2
2G1-P65M	62	47	17	18	28	10	1	2
2G1-P66M	65	48	18	18	26	10	1	1
2G1-P67M	64	47	18	18	25	10	1	1
2G1-P68M	57	42	17	17	25	9	1	2
2G1-P69M	58	42	15	15	23	9	1	1
2G1-P70M	62	46	18	18	26	9	1	2
2G1-P71M	57	43	16	16	23	9	1	2
2G1-P72M	63	45	16	16	25	10	1	1
2G1-P73M	61	45	16	17	25	9	1	1
2G1-P74M	62	47	17	18	25	10	1	1
2G1-P75M	58	45	16	17	24	9	1	2
2G1-P76M	59	43	17	17	26	10	1	2
2G1-P77M	60	43	17	16	23	9	1	2
2G1-P78M	61	48	17	18	24	10	1	2
Vancouver Island, B.C.								
various dates								
2H1-P-1F	67	50	17	18	25	10	1	0
2H1-P-2F	71	53	18	18	24	10	1	0
2H1-P-3F	68	52	18	18	24	10	1	0
2H1-P-4F	68	50	18	18	24	10	1	0
2H1-P-5F	69	49	18	18	25	10	1	0
2H1-P-6F	70	53	19	18	27	10	1	0
2H1-P-7F	68	51	18	18	25	10	1	0
2H1-P-8F	73	55	19	20	28	11	1	0
2H1-P-9F	68	50	18	18	26	10	1	0
2H1-P10F	69	53	18	18	25	10	1	0
2H1-P11F	70	53	18	17	25	11	1	0
2H1-P12F	68	51	19	18	26	10	1	0
2H1-P13F	72	54	17	19	24	11	1	0
2H1-P14F	70	52	18	19	25	10	1	0
2H1-P15F	67	49	18	18	25	10	1	0
2H1-P16F	70	53	18	19	25	10	1	0
2H1-P17F	70	51	19	19	27	10	1	0
2H1-P18F	71	54	17	18	27	10	1	0
2H1-P19F	65	46	17	17	24	10	1	0
2H1-P20F	69	49	18	17	24	10	1	0
2H1-P21F	72	54	19	19	27	11	1	0
2H1-P22F	68	52	18	18	24	10	1	0
2H1-P23F	73	50	17	18	26	10	1	0
2H1-P24F	64	49	17	17	25	10	1	0
2H1-P25F	69	51	18	18	24	10	1	0
2H1-P26F	67	51	18	17	23	10	1	0
2H1-P27F	68	49	18	17	25	11	1	0
2H1-P28F	63	49	18	18	24	9	1	0
2H1-P29F	69	47	18	18	25	10	1	0
2H1-P30F	70	53	19	18	25	10	1	0
2H1-P31F	70	51	19	19	25	10	1	0
2H1-P32F	68	50	19	19	25	10	1	0
2H1-P33F	65	49	17	17	25	11	1	0
2H1-P34F	70	53	18	19	26	11	1	0
2H1-P55F	67	47	18	18	27	10	1	0
2H1-P56F	68	50	15	17	25	9	1	0
2H1-P57F	71	51	17	17	24	10	1	0

Table A5.1, continued.

2H1-P58F	69	51	16	17	23	10	1		0											
2H1-P59F	64	49	18	18	25	10	1		0											
2H1-P60F	68	51	17	17	24	10	1		0											
2H1-P61F	65	46	16	16	25	10	1		0											
2H1-P62F	69	53	18	18	24	10	1		0											
2H1-P63F	67	50	18	19	25	10	1		0											
2H1-P64F	72	53	18	18	25	10	1		0											
2H1-P-1M	61	48	18	17	25	9	1	1	0											
2H1-P-2M	62	48	17	18	24	10	1		0											
2H1-P-3M	60	46	18	18	25	10	1	1	0											
2H1-P-4M	65	50	20	19	26	10	1	1	0											
2H1-P-5M	65	49	19	19	26	11	1	1	0											
2H1-P-6M	63	50	19	19	27	11	1	1	0											
2H1-P-7M	65	48	19	19	26	10	1		0											
2H1-P-8M	65	50	18	19	26	10	1	2	0											
2H1-P-9M	66	49	18	18	25	10	1	1	0											
2H1-P10M	65	49	18	18	25	10	1	1	0											
2H1-P11M	63	47	19	18	26	10	1		0											
2H1-P12M	63	49	18	20	27	10	1	1	0											
2H1-P13M	62	48	18	19	24	10	1	1	0											
2H1-P14M	64	46	19	19	27	10	1	2	0											
2H1-P15M	57	42	17	17	24	10	1	1	0											
2H1-P16M	65	48	19	20	28	10	1	2	0											
2H1-P17M	67	50	19	19	26	10	1	1	0											
2H1-P18M	66	51	19	20	26	11	1	1	0											
2H1-P19M	66	48	19	19	27	11	1	1	0											
2H1-P20M	59	44	17	18	25	10	1	1	0											
2H1-P21M	61	45	17	18	28	11	1		0											
2H1-P22M	62	48	19	18	26	10	1	1	0											
2H1-P23M									1	0	44	52	10	53	0			10.5		
2H1-P24M									1	0	38	49	10	46	0	35	45	11		46
2H1-P47M	64	49	18	17	25	10	1	2	0											
2H1-P48M	66	48	18	18	24	10	1	1	0											
2H1-P49M	64	50	18	17	25	10	1	1	0											
2H1-P50M	65	51	18	18	25	10	1	1	0											
2H1-P51M	63	47	16	16	22	10	1	2	0											
2H1-P52M	62	46	17	18	24	10	1		0											
2H1-P53M	65	48	18	18	25	10	1	1	0											
2H1-P54M	66	48	18	18	25	10	1		0											
2H1-P55M	65	48	18	18	25	10	1	2	0											
2H1-P56M	64	47	19	18	25	10	1	2	0											
2H1-P57M	63	48	19	19	27	10	1	1	0											
2H1-P58M	66	51	18	19	26	10	1	2	0											
2H1P101M									1	0	39	54	9	49	0	43	52	9		50
2H1P102M									1	0	36	50	7	46	1	38	49	7.5		47
Kodiak I., Alaska various dates																				
2I1-P35F	72	50	17	17	23	10	1		0											
2I1-P36F	76	52	18	18	26	10	1		0											
2I1-P37F	69	51	19	18	26	10	1		0											
2I1-P38F	66	48	17	17	23	9	1		0											
2I1-P39F	71	52	18	18	27	10	1		0											
2I1-P40F	69	51	18	17	24	9	1		0											
2I1-P41F	64	48	17	17	24	10	1		0											
2I1-P42F	68	50	18	18	24	10	1		0											
2I1-P43F	68	50	18	18	22	10	1		0											
2I1-P44F	73	49	19	18	25	11	1		0											
2I1-P45F	68	49	17	18	25	9	1		0											
2I1-P46F	67	49	18	18	26	10	1		0											
2I1-P25M	68	53	19	19	26	9	1	1	0											
2I1-P26M	68	49	19	20		10	1	1	0											
2I1-P27M	71	52	20	20	28	10	1	1	0											
2I1-P28M	60	44	18	18	24	9	1	1	0											

Table A5.1, continued.

2I1-P29M	65	51	19	19	25	10	1	1	0											
2I1-P30M	64	47	17	17	25	10	1	1	0											
2I1-P31M	67	47	18	19	26	10	1	1	0											
2I1-P32M	67	47	20	20	26	10	1	1	0											
2I1-P33M	69	48	20	20	28	10	1	1	0											
2I1-P34M	65	48	18	19	25	10	1		0											
P. stygicus																				
Lost Rd. Pond, Alta.																				
VI-28-1989																				
3A2-1F	61	49	18	19	26	9	4		1	0	35	46	6.5	46	0	38	48	7	46	
3A2-2F	60	48	18	20	25	9	5		1	0	34	46	5.5	45	0	36	47	7	46	
3A2-3F	61	46	18	17	25	8	5		1	0	36	46	7.5	45	0	37	46	6.5	44	
3A2-4F	60	45	18	19	26	8	5		1	0	35	48	5.5	42	0	39	49	5	43	
3A2-5F	61	48	19	19	27	9	5		1	0	39	45		47	0	34	45	6.5	44	
3A2-6F	64	48	19	19	24	8	5		1	0	34	47	6.5	49	0	36	45	6	49	
3A2-7F	62	49	19	19	26	9	5		1	0	37	49	6.5	49	0	37	50	7	48	
3A2-8F	60	49	20	19	25	8	5		1	0	31	42	4.5	44	0	32	43	5	42	
3A2-9F	63	52	19	19	26	9	5		1	0	37	47	6.5	48	0	35	49	6.5	49	
3A2-10F	61	46	20	19	25	9	4		1	0	34	47	5.5	48	0	35	47	5	49	
3A2-11F	61	48	16	19	25	8	5		1	0	34	45	6	41	0	34	44	6.5	42	
3A2-12F	59	45	19	19	26	8	5		1	0	34	45	5	45	0	31	42	5	45	
3A2-13F	56	42	17	17	23	8	5		1	0	30	40	5	35	0	29	40	5.5	34	
3A2-14F	57	49	18	18	25	9	5		1						0	34	47	6.5	44	
3A2-15F	64	49	19	19	26	9	5		1	0	38	50	6.5	46	0	39	52	6.5	47	
3A2-16F	60	46	19	19	24	9	5		1	0	38	48		46	0	38	51	4	46	
3A2-P17F	64	48	20	18	25	8	5													
3A2-46F	61	48	19	19	26	8	5		1						a	33	48	7.5	45	
3A2-47F	56	45	19	19	26	8	5		1	0	33	46		43	0	34	44	6.5	42	
3A2-48F	59	49	19	19	26	8	5		1				5.5							
3A2-P50F	57	45	20	19	25	8	5													
3A2-P51F	62	46	18	19	25	8	5													
3A2-18M	57	50	20	20	28	9	5		1	0	29	40		38	0	27	38			
3A2-19M	58	46	19	18	22	8	4		1	0	29	39	3.5	39	0	31	40	3.5	39	
3A2-20M	63	47					4		1	0	33	46	6.5	42						
3A2-21M	65	50	19	19	25	9	5		1	0	35	46	4.5	44	0	34	42	5	44	
3A2-22M	62	48	19	20	27	8	5		1	0	33	46	4.5	43	0	30	44	4.5	43	
3A2-23M	64	50	20	21	28	9	5		1	0	35	43	5	48	0	31	43	5	49	
3A2-24M	60	47	19	20	25	8	5		1	0	32	42	5.5	41	0	34		5.5	41	
3A2-25M	58	47	18	19	25	8	5		1	0	34	44	5.5	42	0	34	44	5	41	
3A2-26M	60	46	18	18	25	8	5		1	0	36	46	5.5	44	0	34	47	5.5	43	
3A2-27M	57	48	19	20	24	8	4		1	0	28	41	6	42	0	28	40	6	41	
3A2-28M	57	45	19	19	26	8	5		1	a	28	39	6	41	a	29	41	6	41	
3A2-29M	59	48	19	20	25	8	5		1	0	31	44	7	42	0	33	43	7	42	
3A2-30M	58	43	19	19	26	9	5		1	0	31	40		38	0	30	41	5.5	38	
3A2-31M	60	47	20	19	25	8	5		1	0	33	44	4.5	39	0	35	44	4.5	41	
3A2-32M	60	48	19	19	24	9	5		1	0	32	45	3.5	43	0	33	44	3.5	43	
3A2-33M	59	44	17	17	24	8	5		1	0	29	38	4.5	37	0			4.5	36	
3A2-34M	58	46	19	20	26	8	5		1	0	31	45	4.5	39	0	35	44	4	41	
3A2-35M	56	47	19	19	26	8	5		1	0	37	46			0			5.5	43	
3A2-36M	59	49	20	20	25	9	5		1	a	37	47	6.5	44	a	37	46	6	40	
3A2-37M	59	47	19	19	27	9	5		1	0			5	42						
3A2-38M	60	47	19	19	26	9	5		1	0	35	44	5	39	0	35	45	5	39	
3A2-39M	62	49	20	20	25	9	5		1	0	36	45	6.5	40	0	37	46	6.5	40	
3A2-40M	57	49	20	19	26	9	5		1	0	34	44	5.5	42				5.5	41	
3A2-41M	60	48	19	19	26		5		1	0	34	44	6.5	45	0	34	44	7	44	
3A2-42M	57	49	19	19	24	8	5		1						0	33		5.5	39	
3A2-43M	52	43	19	19	25	8	5		1						0	28	42	5.5	39	
3A2-44M	51	46	19	20	26	8	5		1						0	33	42	5.5	40	
3A2-45M	61	46	20	21	27	9	5		1						0	34	46	5.5	43	
3A2-P49M	58	45	19	19	26	9	5													
VII-01-1989																				
3A3-1F	59	48	20	19	24	9	5		1	0	34	46	5.5	43	0	32	46	5.5	44	
3A3-2F	60	48	19	20	26	8	4		1	0	41	50	4	46	0	34	47	5	46	

Table A5.1, continued.

3A3-3F	56	44	18	18	25	9	3		1	0	31	41	5.5	45	0	32	42	5.5	44
3A3-P19F	58	45					5												
3A3-4M	61	48	20			9	5		1						0	38	49	6.5	42
3A3-5M	59	47	20	20	27	9	4		1						a	32	42	6	42
3A3-6M	55	43	18	18	24	8	4		1	1	32	44	5	39	0	30	42	6	39
3A3-7M	56	46	20	19	24	9	5		1	0			5	43	0	37	41	6.5	43
3A3-8M	55	43	19	19	26	9	4		1	0	28	39	5	41	0	29		4.5	40
3A3-9M	50	43	19	19	27	9	4		1	0			4.5	44	0	33	42	5	46
3A3-10M	58	47	19	19	26	8	5		1	0	33	41	5	38	0	33	41	5	38
3A3-11M	59	45	19	19	26	8	5		1						0	31	42	6	48
3A3-12M	62	49	19	19	27	9	5		1	0	33	45	5.5	45	0	34		5.5	46
3A3-13M	56	46					5		1									5	41
3A3-14M	60	44	20	20	27	9	4		1						0	33	46	6	46
3A3-15M	59	46	19	20	27	9	4		1	0	32	41	4.5	38	0	31	38	5	40
3A3-16M	61	48	20	20	27	9	5		1	0	34	49	4.5	47	0	34	46	4.5	47
3A3-17M	57	47	20	21	28	8	5		1	0	34	44	5	42	0	35	44	5	40
3A3-18M	60	50	19	20	26	9	5		1	0	36	44	4.5	44	0	34	45	5	45
3A3-P20M	57	46	18	19	25	8	5												
Pecten Pond, Alta.																			
VI-30-1989																			
3B2-1F	60	46	19	19	26	9	5		1	0	36	49	6	45	0	40	48		44
3B2-2F	57	43	18	18	26	8	5		1	0	35	47		47	0	33	46		47
3B2-3F	62	50	19	19	25	9	4		1						0	36	50	7	49
3B2-4F	60	47	19	19	26	8	5		1						0	32	45	6.5	44
3B2-5M	54	44	18	19	25	8	5		1						0	35	47	6	45
3B2-6M	56	46	19	20	26	8	5		1	0	33	42	5	43	0			5	43
3B2-7M	59	47	19	18	28	9	5		1	0	34	45		43	0	35	45	5.5	43
3B2-8M	57	45	19	20	26	8	5		1						0	30	42	4.5	41
Blakiston Ck., Alta.																			
VI-28-1989																			
3C2-1M	62	48	20	20	26	9	5		1						0	34	44	5	42
Antenna Pond, Alta.																			
VII-01-1989																			
3D2-1M	62	47	20	20	27	9	5		1						0	33	44	5	46
Chain Ponds, Alta.																			
VII-11-1987																			
3E1-P60F	60	47	18	19	26	9	4												
3E1-P61F	59	48	18	18	23	8	3												
3E1-P87M	58	44	19	18	25	8	4												
3E1-P88M	58	44	19	19	25	9	4												
3E1-P89M	56	43	19	20	26	9	4	5											
3E1-P90M	58	45	19	19	24	8	4												
3E1-P91M	61	48	20	21	27	9	4												
VII-14-1988																			
3E2-1M	57	43	19	18	24	9	5		1	0	28	41	5.5	43	0	32	42	5	42
VI-11-1989																			
3E3-1F	61	46	19	19	26	8	5		1				5	47	0	35	47	5	45
3E3-2F	63	46	19	19	25	9	5		1	0	32	44	6	47	0	32	46	6	46
3E3-3F	64	50	18	18	24	9	4		1	0	36	48	5	48	0	37	48	6	48
3E3-4F	62	46	18	19	28	9	5		1	0	34	46	5.5	43	0	31	43	5	44
3E3-5F	61	46	18	19	27	9	4		1	0	32	45	7	48	t	33	50	6	47
3E3-6F	61	51	19	19	26	8	4		1	0	33	45	4.5	43	0	36	50	4.5	44
3E3-7F	66	48	20	20	26	9	5		1										
3E3-8F	64	48	20	18	26	9	4		1	0	43	49			0	38	51	5.5	52
3E3-9F			18	18	24	9	5		1	0	34	47	5.5	41	0	34	44	6.5	43
3E3-10F	64	46	19	19	25	9	5		1	0			7	48	0	38	50		
3E3-11F	61	47	18	18	24	9	5		1	0	32	46	5.5	45	0	32	45	5	45
3E3-12F	64	49	21			9	4		1	0	38	49	6.5	52	0	38	51	7.5	53
3E3-102F			18	18	23	9	5		1										
3E3-103F									1										
3E3-13M	58	43	20	20		9	5		1	0	34	42		42					
3E3-14M	56	44	18	19	25	9	5		1	0	28	40	5	36	0	27	41		37
3E3-15M	59	47	19	20	26	9	4		1	0	32	41	5.5	43	0	33	45	5.5	42

Table A5.1, continued.

3E3-16M	57	44					4	1	0	36	45	5	41									
3E3-17M	58	47	19	20	24		5	1	0	40	48	5.5	40	0	39	46	5.5	42				
3E3-18M	60	48	19	19	27	9	5	1	0	33	41	5.5	48	0	33	43	5.5	46				
3E3-19M	56	47	19	19	24	9	5	1	0	31	42		42	0								
3E3-20M	59	43					5	1	0	32	45	6	40	0	30	42	5	40				
3E3-21M	58	45	18	18	25	9	5	1	0	30	38	4.5	42	0	31	41	4.5	40				
3E3-101M			18	18	24	9	5	1														
3E3-104M								1														
VII-01-1989																						
3E4-16F	62	49	18	18	26	9	5	1		32	47	5.5	44	0	35	47	6	44				
3E4-17F	61	49	18	18	25	8	5	1	0	32	44	4.5	34	0	37	42	4.5	44				
3E4-18F	64	48			26		5	1	0	35	44		46	0	31	40	6	47				
3E4-19F	65	49	19	18	25		3	1	0	38	45	6	50	0	37	49	5	49				
3E4-20F	66	48	19	19	26	8	4	1	0	36	47	6.5	47	0	36	44	6.5	49				
3E4-21F	65	50	19	19	25	9	4	1	0			6	47	0	36	50	6.5	47				
3E4-22F	65	50					5	1	t	37	49	5.5	44	t	37	46	5.5	44				
3E4-23F	64	48	19	19	26	9	5	1	0	35	47	6	47	0	35	48	6.5	47				
3E4-24F	62	50	19	19	26	9	4	1	0	37	49	5	46	0	38	50	5	47				
3E4-25F	62	50	18	18	23	9	5	1	0	36	47	5.5	49	0	38	50	5	48				
3E4-26F	60	47	18	18	25	8	5	1				6	44	0								
3E4-27F	67	51	20	20	27	9	5	1	0	34	48	4	55	0	40	52	3.5	54				
3E4-1M	57	46	19	19	25	9	5	1	0	29	41	4.5	44	0	31	43		45				
3E4-2M	60	45	19	18	25	9	5	1						0	34	43	4.5	46				
3E4-3M	54	43	19	19	26	8	4	1	0	29	38	4.5	42	0	34							
3E4-4M	63	47	18	19	24	9	4	1	0	34	47	6	46	0	34	50	6.5	46				
3E4-5M	53	43	18	17	24	8	5	1						0	31	40		40				
3E4-6M	61	48	19	19	24	9	5	1						0	34	44	4.5	44				
3E4-7M	57	46	19	19	24	9	5	1														
3E4-8M	55	44	18	18	25	9	5	1						0	29	40	4.5	39				
3E4-9M	55	44	19	19	25	8	5	1						0	28	39	5	38				
3E4-10M	55	45	19	18	24	9	5	1						0	35	44	4.5	45				
3E4-11M	57	48	19	18	26	8	4	1						0	27	38	6	40				
3E4-12M	60	47	20	20	27	8	5	1						0	33	46	5.5	44				
3E4-13M	59	48	18	18	26	9	5	1	0	32	42	5.5	41	0	38	45	5.5	40				
3E4-14M	61	45	19	19	26	9	5	1	0	25	39	6	43	0	33	45	6.5	44				
3E4-15M	55	45	18	18	22	9	4	1	0	32	39	4	39	0	33	38	5.5	38				
3E4-P28M	58	43					5															
VIII-22-1989																						
3E5-1F	60	51	19	19	26	9	5	1	0			5	48	0	34	46	5	48				
3E5-2F	65	52					5	1	0	38	53	5.5	53									
3E5-3F	65	51					5	1	0	37	47	7	48	0	35	47	5.5	48				
3E5-5F	64	49	19	19	26	8	4	1	0	42	53	5	49	0	39	49	4.5	48				
3E5-6F	56	45	18	19	24	9	5	1	0	29	37	5	37		29	38	5.5	38				
3E5-7F	66	51	20	20	27	9	5	1						0	34	46	5	51				
3E5-8F	65	50	20	20	28	9	5	1						0	37	49	7	49				
3E5-9F	60	48	18	18	24	9	5	1						0	35		6	45				
3E5-11F	64	50	19	18	24	9	5	1		37	51	6.5	48	0	36	47	6	47				
3E5-13F	64	48	18	18	25	8	4	1	0	35	48	6	47	0	34	48	6	47				
3E5-14F	62	50	19	19	25	9	5	1	0	36	48	6	46	0	35	49	5.5	47				
3E5-15F	63	50	19	20	25	9	5	1	0		54	6	47	0	39	50	6.5	49				
3E5-4M	59	47	19	19	25	8	5	1	0	34	44	5	43	0	33	44	4.5	43				
3E5-10M	61	46	19	19	27	9	5	1	0	32	41	4	41									
3E5-12M	59	46	19	19	24	9	5	1						0	32	42	6	42				
3E5-16M	63	48	20	19	25	9	4	1	0	35	45	6.5	47	0	34	45	7					
3E5-17M	60	49	21	20	27	9	5	1		34	44	5	44	0			5.5	48				
3E5-18M	65	52	21	21	28	9	5	1	0	37	49	6	47	0	35	46	6.5	47				
George Lake, Alta.																						
VIII-15-1988																						
3F3-7F	54	41						1														
3F3-24F	59	46						1														
3F3-P26F	60	48	20	20	29	8	5															
3F3-8M	52	43						1														
3F3-10M	59	47						1														

Table A5.1, continued.

V-31-1989																					
3F5-P9F	55	44	17	18	23	8	5														
VI-20-1989																					
3F6-4F			19	19	27	8	4	1													
3F6-5F			19	19	27		5	1													
3F6-6F			18	18	23	8	5	1													
3F6-14F			18	19	24	8	4	1								0	43	53			
3F6-18F			19	19	26	8	5	1													
3F6-19F			16	17	24	8	5	1													
3F6-21F			18	19	24	8	5	1													
3F6-2M			19	19	25	8	5	1													
3F6-3M			19	19	24	9	5	1													
3F6-15M			20	20	25	9	5	1													
3F6-16M			20	19	26	8	4	1													
3F6-20M			18	18	25	8	5	1													
3F6-22M			19	20	25	8	5	1													
3F6-7							5	1													
3F6-8							5	1													
3F6-17			19	19		8	4	1													
VII-06-1989																					
3F7-P37F	58	50	19	19	25	9	5														
3F7-38F	58	45	19	19	25	8	4	1	0	33	50	7	47	0	33	50	7.5	44			
3F7-39F	54	44		18	23		5	1	0	33	47	6	42	0	34	47	6	42			
3F7-41F	58	46	20	20	25	8	5	1	0	38	57	4.5	47	0	38	59	3.5	48			
3F7-P42F	56	43																			
3F7-P43F	54	39	17	17	24	8	5														
3F7-44F	55	45	18	18	25	8	4	1						0	33	48	5	46			
3F7-45F	58	45					5	1						0		44	7	45			
3F7-46F	59	48	19	19	24	8	4	1						0	43	55	5.5	49			
3F7-47F	61	48	20	20	27	8	3	1						0	40	52	7.5	47			
3F7-48F	58	47	18	18	24	8	5	1						0	38	54		47			
3F7-49F	58	46	20	20	26	8	5	1	0	33	47	6	45	0			6.5	45			
3F7-50F	61	47	19	19	25	8	5	1	0	38	56	6.5	50	0	39	53	6	50			
3F7-51F	62	48	19	19	26	9	5	1	0	39	53	9	51	0	37	52	9	51			
3F7-52F	61	46	19	19	27	9	5	1	0	38	51	6.5	48	1	37	50	7	47			
3F7-53F	57	47	18	18	25	9	5	1	0	34	46	5	46	0	36	51	6	48			
3F7-54F	55	47	18	19	25	8	4	1	0	40	52	1	46	0	39	54	2	47			
3F7-55F	58	47	19	19	25	9	4	1	0	37	47	6	47	0	36	47	6	49			
3F7-56F	58	45	18	18	25	8	5	1	0	33	46	5.5	46	0	36	48	6.5	47			
3F7-1M	56	46	19	20	25	8	5	1	0	37	47	5.5	45								
3F7-2M	56	45	20	19	26	9	5	1	0	34	45	6	48	0	36	48	6.5	48			
3F7-3M	51	42	19	19	24	9	5	1													
3F7-4M	55	44	19	19	25	8	5	1				6	42	0	33	45	6	42			
3F7-5M	57	45	20	20	25	9	5	1	0	34		4.5	42	0	33	47	4.5	45			
3F7-6M	56	46	19	19	24	9	5	1	0	38	46	6.5	44	0	32	47	7	44			
3F7-7M	59	45	19	20	26	9	5	1	0	32	45	6	41	0	35	46	6	42			
3F7-8M	55	44	19	20	25	9	5	1	0	32	47	6	43								
3F7-9M	60	44					5	1	0	30	41	7	43								
3F7-10M	58	47	19	19	25	9	5	1						1	38	50	6.5	46			
3F7-11M	55	43	18	19	25	8	5	1	0	32	43	6	42								
3F7-12M	57	44	20	19	26	8	5	1						0	37	49	2	44			
3F7-13M	55	46	19	19	26	9	5	1	0	34	48	6	44	0	32	45	6.5	43			
3F7-14M	55	42	19	19	24	9	5	1	0			4.5	38	0	32	45	5	39			
3F7-15M	59	48	19	19	25	9	5	1	0	37	53	5.5	49	0	38	52	6.5	50			
3F7-16M	61	48	20	19	26	8	5	1	0	33	46	6.5	47	0	33	45	7.5	47			
3F7-17M	54	44	18	19	24	8	5	1	0	34	46		41	0	33	49	7	41			
3F7-18M	53	49	19	19	25	8	5	1	0	29	47	4.5	45	0	29		4.5	44			
3F7-19M	58	45	18	19	25	8	5	1	0	31	42	6	41	0	29	44	6	39			
3F7-20M	51	44	19	19	25	8	5	1	0	29	43	5.5	41				5	39			
3F7-21M	57	45	19	19	26	8	5	1	0	33	44	7						42			
3F7-22M	58	46					4	1	0	35	46	6.5	44	0	37	48	6.5	44			
3F7-23M	54	44	20	18	25	8	4	1	0	32	43	6.5	43	0	33	46	6.5	42			
3F7-24M	57	46	20	20	25	8	5	1	0	34	49	6	45	0	34	50	5	46			

Table A5.1, continued.

3F7-25M	57	46	20	20	25	8	5		1											
3F7-26M	56	43	18	19	25	8	5		1											
3F7-27M	55	44	19	19	25	8	5		1	0			6.5	42	0	30	45	6.5	41	
3F7-28M	56	44	19	19	25	8	4		1	0	29	46	6.5	43	0	30	45		43	
3F7-29M	58	45			25	8	5		1											
3F7-30M	58	44			25	8	5		1											
3F7-31M	55	43	18	19	25	8	4		1	0	32	43	4.5	42	0	32	42	5	40	
3F7-32M	55	45	20	20	26	8	5		1	0	36	49	5	47	0	36	47	5	47	
3F7-33M	55	43	19	18	24	8	4		1	t				40	t	32	42	7	39	
3F7-34M	62	48	19	20	27	8	5		1											
3F7-35M	58	46	20	19	24	8	5		1											
3F7-36M	60	46	19	19	27	8	5		1											
3F7-P40M	61	46	19	18	24	8	5													
3F7-P57M	53	43	19	19	25	8	5	5												
VII-20-1989																				
3F8-1M	57	45	20	20	27	8	5		1											
VIII-09-1990																				
3F9-2F	57	47							1											
3F9-3F	63	49							1											
3F9-1M	55	43							1											
Long Lake Pond, Alta.																				
VI-22-1988																				
3G2-P12F	59	48	19	19	25	8	5													
3G2-P13F	61	48	19	19	26	9	5													
3G2-P15F	60	46	18	19	25	9	5													
3G2-P17F	63	50	19	19	27	8	5													
3G2-P11M	54	42	18	18	26	8	5													
3G2-P14M	59	47	19	20	25	8	5													
3G2-P16M	53	42	20	20	25	8	5													
3G2-P18M	58	45	20	19	26	8	5													
VII-27-1988																				
3G3-14F							4		1						0	33	50	8	48	
3G3-18F							4		1						0	34	55			
3G3-20F									1	0	36	50				27		7.5	50	
3G3-22F							5		1						0	34	51	6.5		
3G3-1M							5		1						0	30	43		41	
3G3-5M			19	18	25	8	5		1											
3G3-6M			20	20	27		5		1											
3G3-7M							4		1	0	36	47	5.5	47	0	35	50	6	48	
3G3-9M							5		1	0	35		4.5	44	0	32	46	6.5	44	
3G3-11M							5		1					42				4.5	43	
3G3-13M			19	19	25	8	5		1											
3G3-23M			19	19	27	9	5		1											
3G3-24M			20	20	27	8	5		1											
VIII-11-1988																				
3G4-7	59	44	19	19	25	8	5		1	t	33	46	6	44	0	30	45	7	44	
VII-07-1989																				
3G5-1F	57	47	19	19	25	9	3		1	0	36	51	7.5	47	0	35	49	7	47	
3G5-2F	63	50	18	18	25	9	4		1	0	37	47	6	49	a	39	49	7.5	47	
3G5-9F	61	49							1											
3G5-11F	60	48	18	18	24	8	5		1	0	36	53	6.5	47	0	36	50	6	46	
3G5-3M	58	46	19	19	24	8	5		1	0	37	49	0	48	0	42	50	7	48	
3G5-4M	60	48	20	21	27	8	5		1	0			6	44	0			5.5	46	
3G5-5M	60	46	20	20	27	9	5		1	0	33	44	5.5	41	0	34	45	6.5	41	
3G5-6M	52	42	19	19	25	8	5		1	0	33	43	6	43	0	33	44	5	42	
3G5-7M	52	45	20	20	25	9	5		1	0	34	45	6.5	45	0	34	46	6	44	
3G5-8M	59	47							1											
3G5-10M	58	48	20	20	25	8	4		1	0	35	51	1.5	52	0	36	52	5.5	52	
Hinton, Alta.																				
VII-11-1989																				
3H2-7F	60	49	19	19	25	8	5		1											
3H2-8F	61	47	18	18	26	9	4		1											
3H2-9F	59	47	18	18	24	8	5		1	d	36	48	7.5	49	0	38	46	7.5	48	

Table A5.1, continued.

3H2-10F	62	49	19	19	26		5	1											
3H2-11F	60	46	19	19	24	8	5	1											
3H2-1M	56	45	19	19	23	8	5	1											
3H2-2M	56	47					5	1											
3H2-3M	58	47	19	18	26	9	5	1											
3H2-5M	52	43	18	18	23	8	5	1											
VII-31-1989																			
3H3-3F	60	50	18	18	25	8	4	1	0	35	51	7.5	48	0	36	48	7	48	
3H3-4F	56	45	18	18	25	8	5	1	0	35	46	5.5	44	0	37	46	4.5	46	
3H3-6F	62	50	19	19	26	9	5	1	t	37	54	8	51	t	35	52	8	52	
3H3-10F	67	53	19	19	27	9	5	1	0			9	56	0	43	55	9	56	
3H3-11F	61	49	19	20	27	9	4	1	0	40	51	7.5	51	0	39	52	6.5	50	
3H3-16F	60	49	18	18	25	8	5	1	0	35	46	6.5	48	0	36	48	6	47	
3H3-17F	63	50	19	18	26	9	5	1	0	38	52	7.5	49	0	37	50	7	51	
3H3-18F	57	47	18	18	25	8	4	1	0	36	50	5.5	45	0	36	49	5	46	
3H3-1M	61	47	20	20	27	9	5	1					41	0	34	51	6.5	46	
3H3-2M	59	45	19	19	25	8	5	1	0	34	46	4.5	41	0	33	46	5	43	
3H3-5M	57	43	19	19	27	8	5	1	d	30	41	3.5	40	d	30	42	5.5	41	
3H3-9M	54	43	18	18	25	8	5	1	0					0	27	37	5		
3H3-12M	51	47	19	19	26	9	5	1	t	33	43	3.5	46	t	34	44	5	46	
3H3-13M	59	48	19	19	26	8	5	1	0	35	42	7	47	0	32	50	7.5	48	
3H3-14M	58	49	17		25	9	5	1	0	37	49	5	49	0	37	50	5	49	
3H3-19M	53	47	19	19	24	8	4	1	0	33	46	6	45	0	34	44	6	43	
Lynch Lakes, Alta.																			
VII-01-1989																			
3I2-9F	68	49	19	19	25	9	4	1	0	37	49	6	49	0	38	48	6	48	
3I2-10F	59	48	19	18	25	8	5	1	0	31	46	6.5	45	0	32	44	6.5	47	
3I2-23M	62	47	19	19	26	9	4	1	0	34	46	5	47	0	34	48	4.5	47	
3I2-24M	57	48	19	19	25	8	5	1	0	36	50	6	45		37		5.5	43	
3I2-25M	60	45	19	19	26	9	5	1	0	33	48	6.5	40	0	33	46	5.5	40	
3I2-26M	60	48	20	21	27	9	5	1	0	31	45		44	0	32	44	5	43	
3I2-27M	58	46	19	19	25	8	5	1	0	33	44		40	0	34	46	3.5	40	
VII-20-1989																			
3I3-15F	59	49	19	19	24	8	5	1	0	36	50	6	46	0	36	48	6	45	
3I3-17F	57	47	18	18	25	9	4	1	0			5	39	0	28	40		39	
Wauconda, Washington																			
VII-18-1989																			
3J2-12F	58	46	17	17	22	8	5	1	0	32	45	8	40	0	30	40	7	38	
3J2-15F	59	44	16	18	24	8	5	1	0	28	39	6.5	40	0	29	40	6.5	39	
3J2-28F	60	46	17	17	24	8	5	1	0	31	44	7	43	0	32	45	6.5	42	
3J2-18M	55	45	18	18		8	4	1											
3J2-19M	64	44	19	19	25	9	5	1	0	31	45	6	41	0	33	44	5.5	40	
3J2-22M	56	42	17	17	24	8	5	1	0	29			38		29			38	
3J2-24M	57	45	18	19	24	8	4	1	0	28	38	5	38	0	28	41	5	39	
3J2-27M	59	46	18	19	25	8	5	1	0	29	41	7	41	0	27	41	6.5	40	
3J2-29M	59	46	18	19	25	9	4	1		30	44	6	40	0	30	40	5	40	
Circle, Alaska																			
VII-3+4-1958																			
3K1-P-1F	56	45	17	18	24	8	5	1											
3K1-P-2F	54	43	17	16	24	8	4	1											
3K1-P-3F	61	47	19	18	25	9	4	1											
3K1-P-4F	63	48	17	17	22	9	4	1											
3K1-P-5F	55	45	17	18	23	9	3	1											
3K1-P-6F	56	43	17	18	24	8	5	1											
3K1-P-7F	63	48	18	17	25	9	4	1											
3K1-P-8F	59	47	18	18	24	9	4	1											
3K1-P-9F	62	47	18	18	24	8	4	1											
3K1-P10F	61	49	17	17	25	9	5	1											
3K1-P11F	61	48	17	17	23	9	5	1											
3K1-P12F	57	46	17	18	25	9	4	1											
3K1-P13F	63	50	19	19	25	9	4	1											
3K1-P14F	62	48	18	19	25	9	4	1											
3K1-P15F	54	44	16	17	23	8	4	1											

Table A5.1, continued.

3K1-P16F	59	48	17	17	24	8	4		1
3K1-P-1M	59	45	18	18	24	9	4		1
3K1-P-2M	56	45	17	17	24	9	4	5	1
3K1-P-3M	54	42	17	17	24	9	5	4	1
3K1-P-4M	54	46	17	17	23	9	4	5	1
3K1-P-5M	58	47	19	18	26	9	4	5	1
3K1-P-6M	58	47	19	19	24	9	4	4	1
3K1-P-7M	54	42	18	17	22	8	3	5	1
3K1-P-8M	58	47	18	18	25	9	4	5	1
3K1-P-9M	57	45	18	18	25	9	3	5	1
3K1-P10M	56	46	17	17	25	9	4	5	1
3K1-P11M	59	46	17	18	23	9	3		1
3K1-P12M	58	44	18	18	24	8	4		1
3K1-P13M	60	48	18	18	24	9	4		1
3K1-P14M	60	47	18	18	26	9	3		1
3K1-P15M	56	46	18	18	25	9	3		1
3K1-P16M	57	45	18	19	25	8	3	5	1
3K1-P17M	59	49	18	18	24	9	3		1
3K1-P18M	56	44	18	18	25	9	4		1
3K1-P19M	58	47	17	18	25	9	3		1
3K1-P20M	57	46	17	17	25	8	4		1
3K1-P21M	54	46	17	17	25	8	4		1
3K1-P22M	56	43	18	17	24	8	4		1
3K1-P23M	57	46	19	18	24	8	3		1
3K1-P24M	57	47	19	18	26	9	4		1
3K1-P25M	57	47	17	18	24	9	3		1
3K1-P26M	57	46	18	17	23	9	3		1
Williams Lake, B.C.									
VI-05-1958									
3L1-P17F	65	50	19	19	26	9	4		1
3L1-P18F	64	50	18	18	26	9	4		1
3L1-P19F	61	46	19	19	26	9	3		1
3L1-P20F	58	47	18	19	24	9	3		1
3L1-P21F	57	46	18	17	24	8	4		1
3L1-P22F	62	49	19	19	26	9	4		1
3L1-P23F	62	48	18	17	26	9	4		1
3L1-P24F	65	49	18	19	26	9	4		1
3L1-P25F	59	48	18	18	23	9	5		1
3L1-P26F	64	49	19	19	25	9	4		1
3L1-P27M	56	46	18	18	26	9	4	5	1
3L1-P28M	58	47	18	19	27	9	4	5	1
3L1-P29M	54	44	18	18	26	9	4	5	1
3L1-P30M	61	48	19	19	27	9	4	5	1
3L1-P31M	60	47	18	18	25	9	4		1
3L1-P32M	61	47	19	20	27	9	4	5	1
3L1-P33M	59	49	19	19	26	9	4		1
3L1-P34M	58	47	19	20	27	9	4	5	1
3L1-P35M	56	42	19	19	26	9	3		1
3L1-P36M	60	47	18	18	26	9	4	5	1
3L1-P37M	60	47	19	19	26	9	4	5	1
3L1-P38M	60	46	20	20	25	9	3	5	1
3L1-P39M	60	48	19	19	25	9	4	5	1
3L1-P40M	59	48	19	19	25	8	3		1
3L1-P41M	60	48	21	20	27	9	4		1
Cypress Hills, Alta./Sask.									
various dates									
3N1-P48F	58	47	19	19	26	9	4		
3N1-P49F	63	50	18	18	25	9	4		
3N1-P50F	59	47	18	18	25	9	4		
3N1-P51F	61	49	19	19	26	9	4		
3N1-P52F	55	46	18	17	24	9	4		
3N1-P53F	59	46	19	19	25	8	4		
3N1-P54F	62	48	19	19	26	9	4		

Table A5.1, continued.

3N1-P55F	60	48	18	18	25	9	4	
3N1-P56F	61	49	20	20	28	9	3	
3N1-P57F	63	52	20	20	27	9	4	
3N1-P58F	62	47	18	18	26	10	4	
3N1-P59F	64	51	19	20	27	9	3	
3N1-P60F	62	47	19	20	26	8	3	
3N1-P61F	58	45	18	18	24	8	3	
3N1-P62F	67	51	20	20	27	9	4	
3N1-P63F	59	48	18	18	24	9	4	
3N1-P64F	58	45	17	19	25	9	3	
3N1-P70M	59	48	19	19	26	9	3	
3N1-P71M	58	46	20	21	27	8	4	5
3N1-P72M	61	47	19	20	27	9	4	5
3N1-P73M	60	47	20	20	26	9	3	5
3N1-P74M	60	47	20	19	27	8	3	5
3N1-P75M	64	48	19	19	25	10	3	3
3N1-P76M	62	49	19	18	25	9	3	4
3N1-P77M	58	47	18	18	26	9	4	5
3N1-P78M	56	44	19	19	26	8	3	5
3N1-P79M	58	44	19	19	25	9	4	5
3N1-P80M	58	46	19	19	26	8	3	5
3N1-P81M	59	46	18	19	26	9	3	5
3N1-P82M	55	43	19	19	26	9	3	
3N1-P84M	53	40	16	16	22	8	3	4
3N1-P85M	57	45	19	19	25	9	3	5
3N1-P86M	54	43	19	19	25	9	3	5
3N1-P87M	60	47	19	18	24	9	3	
3N1-P88M	58	45	19	19	26	9	3	5
3N1-P89M	63	50	20	20	28	9	3	4
3N1-P90M	57	48	21	20	24	8	4	5
3N1-P91M	62	48	19	20	25	10	4	4
3N1-P92M	60	50	20	20	26	9	3	4
3N1-P93M	56	44	19	18	25	9	3	5
3N1-P94M	57	46	19	19	26	9	4	5
3N1-P95M	62	50	20	20	26	9	4	5
3N1-P96M	59	46	20	19	24	9	4	5
3N1-P99M	55	44	18	18	22	9	3	5
Cranbrook, B.C. various dates								
3O1-P-1F	60	46	18	18	23	8	4	
3O1-P-2F	61	46	19	19	24	9	4	
3O1-P-3F	63	49	19	19	25	8	4	
3O1-P-4F	65	52	18	18	25	9	3	
3O1-P-5F	64	50	18	19	26	9	4	
3O1-P-6F	62	47	17	18	24	8	4	
3O1-P-7F	64	50	18	18	27	9	5	
3O1-P-8F	64	50	19	20	25	9	3	
3O1-P-9F	63	48	20	19	27	9	4	
3O1-P10F	59	46	19	21	26		3	
3O1-P11F	61	45	19	20	27		4	
3O1-P12F	64	49	19	20	26		4	
3O1-P13F	67	52	19	19	26	9	4	
3O1-P14F	63	49	19	19	25	9	4	
3O1-P15F	68	51	19	20	27	9	4	
3O1-P16F	60	46	19	19	25	8	4	
3O1-P17F	67	49	19	20	25	9	4	
3O1-P18F	68	52	19	19	25	9	4	
3O1-P19F	60	45	18	19	26	9	4	
3O1-P20F	65	50	19	19	26	9	4	
3O1-P21F	64	49	20	20	26	9	4	
3O1-P22F	62	47	20	20	25	9	4	
3O1-P23F	65	51	20	20	26	9	3	
201-P24F	64	49	18	18	25	9	4	

Table A5.1, continued.

3O1-P25F	62	47	18	19	26	8	3	
3O1-P26F	65	49	19	19	27	9	3	
3O1-P27F	65	49	18	19	25	9	4	
3O1-P28F	64	49	19	19	26	9	4	
3O1-P29F	66	50	19	20	27	9	4	
3O1-P30F	64	50	20	20	26	8	4	
3O1-P-1M	66	48	19	19	25	9	4	4
3O1-P-2M	61	48	21	21	28	9	4	4
3O1-P-3M	65	50	20	20	27	9	4	4
3O1-P-4M	60	48	19	20	26	9	4	4
3O1-P-5M	59	47	19	20	26	9	4	4
3O1-P-6M	63	49	19	19	26	10	4	4
3O1-P-7M	60	46	19	19	25	8	4	5
3O1-P-8M	61	50	20	19	26	9	4	4
3O1-P-9M	60	46	19	19	26	9	4	4
3O1-P10M	61	46	19	20	26	9	4	4
3O1-P11M	61	46	19	17	24	9	4	4
3O1-P12M	65	50	21	21	28	9	4	4
3O1-P13M	61	46	19	19	27	10	4	5
3O1-P14M	59	47	20	19	26	9	4	5
3O1-P15M	60	46	18	18	26	9	4	4
3O1-P16M	59	47	19	19	26	9	4	5
3O1-P17M	64	50	20	19	26	9	4	5
3O1-P18M	63	50	21	20	28	11	4	4
3O1-P19M	62	48	20	20	26	9	4	4
3O1-P20M	61	49	19	21	27	10	4	5
3O1-P21M	57	47	20	19	27	9	4	4
3O1-P22M	59	45	19	19	25	9	4	4
3O1-P23M	59	45	18	18	25	9	4	4
3O1-P24M	61	47	19	19	25	9	4	5
3O1-P25M	59	46	20	20	26	9	4	5
3O1-P26M	61	46	18	19	24	9	4	4
3O1-P27M	63	46	19	19	25	8	3	5
3O1-P28M	58	46	19	19	26	9	4	4
Columbia Lake, B.C.								
VII-31-1956								
3P1-P41F	54	44	18	18	24	8	4	
3P1-P42F	61	47	18	18	27	8	3	
3P1-P43F	62	48	18	20	25	9	3	
3P1-P44F	58	45	18	19	24	8	3	
3P1-P45F	59	46	18	18	24	9	3	
3P1-P46F	62	48	19	19	25	9	3	
3P1-P55M	62	48	20	19	27	9	3	5
3P1-P56M	61	48	20	20	27	9	4	4
3P1-P57M	63	47	20	20	27	9	3	5
3P1-P58M	63	49	19	19	26	9	3	5
3P1-P59M	58	45	19	18	25	8	4	5
3P1-P60M	57	44	20	19	25	9	3	5
3P1-P61M	54	43	17	18	24	8	3	5
3P1-P63M	59	47	19	19	25	9	3	5
Wardner, B.C.								
VII-27-1956								
3Q1-P55F	62	50	18	19	26	9	4	
3Q1-P56F	62	48	20	19	26	9	3	
3Q1-P57F	62	51	19	19	26	9	4	
3Q1-P58F	60	45	18	20	24	9	3	
3Q1-P80M	58	46	18	20	25	9	3	5
3Q1-P81M	56	43	19	19	25	8	4	5
Kavanaugh's Pond, Alta.								
VII-18-1970								
3R1-P-1F	63	48	19	19	26	9	5	
3R1-P-2F	62	47	19	19	26	9	4	
3R1-P-3F	58	46	19	20	25	8	5	

Table A5.1, continued.

3R1-P-4F	62	48	19	18	24	8	5													
3R1-P-5F	63	44	18	19	25	9	4													
3R1-P-6F	60	43	18	19	25	8	5													
3R1-P-7F	65	50	18	19	27	9	5													
3R1-P-8F	56	44	18	18	24	9	5													
3R1-P-9F	64	48	20	20	27	9	5													
4B1-P-1F	59	45	18	19	26	9	3		1	0	31	43	6	43	0	32	43	6	42	
3R1-P-1M	57	47	20	20	27	9	5	4												
3R1-P-2M	61	47	20	20	26	9	5	5												
3R1-P-3M	62	46	20	20	26	9	4	5												
3R1-P-4M	60	45	19	20	26	9	4	5												
3R1-P-5M	60	45	19	19	26	8	5	5												
3R1-P-6M	57	42	19	19	27	8	5	5												
3R1-P-7M	61	47	20	19	26	9	5	4												
3R1-P-8M	57	49	20	19	27	8	5	5												
Kananaskis, Alta.																				
VI-02-1969																				
3S1-P-3F	56	46	17	18	23	8	5													
3S1-P-1M	58	47	19	19	25	8	5													
3S1-P-2M	57	45	20	20	26	9	5	5												
3S1-P-4M	55	45	18	19	25	8	5	5												
Bull Pond #2, Alta.																				
VII-12-1988																				
3T1-P-1F	61	44	20	19	25	9	5		1	0	35	50	6	49	0	36	50	4.5	49	
Island Pond, B.C.																				
VIII-28-1955																				
3V1-P84M	62	48	18	19	26	10	3	5												
P. fossifrons/stygicus hybrids																				
Wauconda, Washington																				
VII-18-1989																				
4A2-20F	73	54	18	18	25	9	2		0											
Rock Pond, Alta.																				
VI-29-1989																				
4D2-P55M	70	50	22	21	27	10	5	3	0											
Ball's Pond, Alta.																				
VII-26-1956																				
4E1-102M	67	49	22	22	30	9	5	4	0											

VITA

Name: Gregory Ray Pohl

Place of Birth: Edmonton, Alberta

Year of Birth: 1965

Post-Secondary Education:

B.Sc. (Specialization in Entomology), University of Alberta, 1987.

M.Sc. (Entomology), University of Alberta, 1992.

Honours and Awards:

Natural Sciences and Engineering Research Council of Canada Postgraduate Scholarship (1988 and 1989).

Entomological Society of Canada Postgraduate Student Awards (1989-90).

Current Position (as of September 1991):

Biological Control Technician, Agriculture Canada Research Station, Lethbridge, Alberta.

Publications:

LANGOR, D.W., SPENCE, J.R., and POHL, G.R., 1990. Host effects on fertility and reproductive success of Dendroctonus ponderosae Hopkins (Coleoptera: Scolytidae). Evolution 44(3): 609-618.